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Health and Environmental Effects Profile for Benzidine

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR BENZIDINE

ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
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16. ABSTRACT

The Health and Environmental Effects Profile for Benzidine was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates have been presented provided sufficient data are available. Benzidine has been evaluated as a carcinogen. The human carcinogen potency factor (q_1^*) for benzidine is 234.13 $(mg/kg/day)^{-1}$ for oral exposure.

The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified by CERCLA based on chronic toxicity. The RQ value for benzidine is 100.

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PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched."

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include, Acceptable Daily Intakes (ADIs) for chronic exposures. An ADI is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, ADIs are not estimated in this document series. Instead, a carcinogenic potency factor or q_1^* is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA docket.

ACKNOWLEDGEMENTS

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EXECUTIVE SUMMARY

Benzidine is a crystalline compound that had been important in commerce because of its usefulness as an intermediate in the manufacturing of azo dyes. All large-scale production of benzidine as an endproduct was terminated in the United States in 1976; however, its production as a nonisolated intermediate (as the dihydrochloride salt) has continued by U.S. dye manufacturers (Ferber, 1978; IARC, 1982). From the list of dyes commercially derived from benzidine (Lurie, 1964), a total of 11 dyes were commercially produced in the United States in 1984 by two dye manufacturers (USITC, 1985). In comparison to the use of benzidine as an azo dye intermediate, the amount of benzidine consumed in all other applications is insignificant (IARC, 1982). Most of the reported minor uses of benzidine (IARC, 1982; Shriner et al., 1978) involve its use as an analytical detection agent.

Oxidation is probably the most important fate process for benzidine in water. Benzidine is rapidly oxidized by metal cations (U.S. EPA, 1981) and other cations that are found in natural waters (Callahan et al., 1979). In addition, oxidation by peroxy radicals in sunlit waters occurs at a rapid rate (Mabey et al., 1981) with a calculated half-life of 6.3 hours. Benzidine can also be oxidized in water by direct reaction with oxygen (U.S. EPA, 1981; Callahan et al., 1979). Photosensitized photolysis of benzidine or photolysis of benzidine-oxidation complexes in water may also have some importance. Biodegradation in natural water is not expected to be a significant process. Benzidine is strongly adsorbed by clay particles (Callahan et al., 1979; Furukawa and Brindley, 1973; Lahav and Raziel, 1971; U.S. EPA, 1981) and by sediment (Zierath et al., 1980), which suggests the importance of adsorption in aquatic environments. Hydrolysis and volatilization from

water are not environmentally important (Mabey et al., 1981; Callahan et al., 1979; U.S. EPA, 1981). Experimentally determined BCFs of 44, 83 and 110 have been reported in fish (Shriner et al., 1978; Freitag et al., 1984; Lu et al., 1977). A persistence study conducted with a single lake water found benzidine to have a half-life of 4 hours (Howard and Saxena, 1976).

In the atmosphere, benzidine is probably removed quite rapidly by reaction with OH• radicals. Based on reaction kinetics (Atkinson, 1985) of structurally similar compounds (aniline and biphenyl), the half-life for the benzidine reaction with OH• radicals is ~1 day. Photolysis and direct oxidation with oxygen may also contribute to the removal of benzidine from air.

Benzidine is rapidly oxidized by clay to a blue complex (Callahan et al., 1979; Lahav and Raziel, 1971). This reversible oxidation is stabilized by adsorption to the clay (Zierath et al., 1980). In the absence of clay or other suitable sorbents, benzidine is irreversibly oxidized to a brown degradation product (Zierath et al., 1980). The UV absorption spectrum for benzidine and the benzidine-clay oxidation complexes (Lahav and Raziel, 1971) and the significant photolysis of benzidine adsorbed to silica gel (Kotzias et al., 1981) suggest that photolysis on sunlit soil surfaces is likely to occur. Based on sorption coefficients in 14 different soils and sediments (Zierath et al., 1980), benzidine is not expected to leach significantly in most types of soil.

Benzidine has been detected in the effluents from factories using benzidine-derived dyes (IARC, 1982), in river water downstream from dye factories (Takemura et al., 1965) and in other industrial effluents (U.S. EPA, 1981). Although benzidine-derived dyes contain residual amounts of

benzidine (Boeniger et al., 1981), the presence of benzidine in certain waters receiving dye wastes may be the result of the reduction of the dye to yield benzidine as a decompostion product (Takemura et al., 1965); the degradation of a benzidine dye has been shown to yield benzidine (Yoshida et al., 1973). Benzidine has not been detected in food or in ambient atmosphere.

Benzidine is acutely toxic to fishes at concentrations as low as 2.5 mg/ℓ, the 96-hour LC_{50} for the red shiner (U.S. EPA, 1980a). A concentration of 1 mg/ℓ has been reported to cause sublethal effects in sheepshead minnows (Martin, 1982) and Daphnia sp. (Klein et al., 1984). Benzidine is not strongly bioaccumulated by aquatic organisms or biomagnified in aquatic food chains (Lu et al., 1977).

Benzidine is absorbed through the skin (Meigs et al., 1951; Barsotti and Vigliani, 1952), by the gastrointestinal tract and the lung (Ghetti, 1960). Radioactivity was rapidly distributed to tissues of dogs, monkeys and rats within 1 hour of intravenous dosing with ^{14}C-benzidine (Kellner et al., 1973). Blood elimination curves were multiphasic, and low residue levels were detectable 7 and 14 days after dosing. Following parenteral administration of ^{14}C-benzidine to rats and dogs, radiolabel was found in bile and was accumulated in the gastrointestinal tract (Kellner et al., 1973; Lynn et al., 1984; Engelbertz and Babel, 1953). Benzidine metabolites vary considerably among species (U.S. EPA, 1980a). Benzidine is acetylated and hydroxylated in humans (Englebertz and Babel, 1956; Troll et al., 1963; Sciarini and Meigs, 1961a). Dogs do not acetylate benzidine appreciably, while rats readily acetylate and conjugate the compound (Lynn et al., 1984). The metabolites produced in rats are dose-dependent (Lynn et al., 1984).

Several recent in vitro metabolism studies were located. Rats predominantly excrete benzidine and its metabolites in the feces (Kellner et al., 1973; Lynn et al., 1984), while dogs and monkeys excrete benzidine and its metabolites primarily in urine, although fecal excretion is also appreciable (Kellner et al., 1973). Elimination curves for urine and feces of dogs and rats are multiphasic (Kellner et al., 1973), which (along with the findings of radioactivity in bile and the gastrointestinal tract after parenteral dosing with ^{14}C-benzidine, the long residence time in the liver, tissues and carcass, and the high degree of conjugation and fecal excretion) indicates that biliary excretion and enterohepatic recirculation occur extensively.

The carcinogenicity of benzidine has been studied in numerous bioassays in a variety of mammalian species with consistently positive results. Dose-related increased incidences of hepatocellular carcinomas were observed in male and female mice exposed to benzidine dihydrochloride in the drinking water at levels $\geq$20 ppm for up to 33 months (Littlefield et al., 1983, 1984; Frith et al., 1979, 1980). Female mice were more susceptible than male mice. Higher incidences of Harderian gland tumors in both sexes and uterine angiomas in female mice were also observed at all drinking water levels (Littlefield et al., 1983). Liver tumors in mice exposed to benzidine dihydrochloride in drinking water at 300 or 500 ppm were observed as early as 39 weeks of treatment (Frith and Dooley, 1976). Dose-related increased incidences of liver and Harderian gland tumors were also observed in mice exposed to benzidine dihydrochloride in the diet at 50-150 ppm for 90 weeks (Vesselinovitch et al., 1975). Mice gavaged twice weekly with doses equivalent to the 50 and 100 ppm dietary levels developed fewer liver and Harderian gland tumors than did the mice exposed to benzidine continuously

in the diet. Higher incidences of liver tumors were found in mice when the onset of treatment occurred during infancy than during adulthood. Male offspring of female mice exposed to dietary benzidine dihydrochloride during gestation or lactation had increased incidences of liver tumors, while female offspring did not (Vesselinovitch et al., 1979). Exposure of mouse dams during gestation and lactation, however, resulted in increased incidences of liver tumors in both male and female offspring, but the incidences were higher in males than in females. Thus, male mice appear to be more susceptible than females to the hepatocarcinogenicity of benzidine when exposed early in life, while female mice are more susceptible than males when exposure occurs postweaning.

Dose-related increased incidences of mammary gland tumors were observed in female rats given 10 doses of 12-50 mg/rat benzidine by gavage over 30 days and observed for 9 months (Griswold et al., 1963). Male and female rats exposed by inhalation to 10-20 mg/m³ benzidine, 4 hours/day, 5 days/week for 28 months also developed mammary gland tumors as well as myeloid leukemia (Zabezhinskii, 1970). Hamsters given benzidine or benzidine dihydrochloride in the diet at 1000 ppm had high incidences of benign and malignant cholangiomas and hepatomas, while hamsters maintained on control diets had no liver tumors (Saffiotti et al., 1967; Sellakumar et al., 1969). Three of six mongrel female dogs treated orally with benzidine in capsules at a TWA dose of 236 mg/kg/day for 60 months and observed for at least 10 years developed urinary bladder cancer (Spitz et al., 1950; Spitz, n.d.). No bladder tumors, however, were found in dogs fed a total of 700 g of benzidine over 5 years (Bonser et al., 1956), 1.0 mg/kg, 5 days/week for 3 years (Deichmann et al., 1965) or 5 mg/kg/day for 6 years (Marhold et al., 1967). The results indicate that while benzidine may induce bladder cancer in dogs, a long latency period is required.

Numerous epidemiology and case studies have demonstrated that occupational exposure to benzidine results in bladder cancer in humans. Zavon et al. (1973) found 13 cases of bladder cancer in 25 workers exposed to workroom levels of benzidine of <0.005-17.6 mg/m³ for a mean of 11.46 years. Based on mean urinary levels of benzidine at the end of the workshift, U.S. EPA (1980a) estimated a mean total accumulated dose of 130 mg/kg in the workers. Tsuchiya et al. (1975) reported 72 cases of bladder cancer in 1015 workers engaged in the production or use of benzidine for ≤23 years. Exposure levels were not quantitated. Excess cases of bladder cancer attributable to benzidine exposure have also been reported by Case et al. (1954), Mancuso and El-Attar (1966, 1967), Yamaguchi et al. (1982), Morinaga et al. (1982), Budnick et al. (1984), Segnan and Tanturri (1976), Qi et al. (1983) and Goldwater et al. (1965), among others.

Benzidine has been widely studied for mutagenicity and genotoxicity in bacterial and yeast systems, in cultured mammalian cell systems and in rodents *in vivo*. Results were generally positive. Although pertinent data regarding the teratogenicity of benzidine to laboratory animals were not available, Vesselinovitch et al. (1979) observed an increased incidence of hepatocellular carcinomas in the male, but not the female, offspring of female mice maintained on a diet containing 150 ppm benzidine dihydrochloride during gestation. Budnick et al. (1984) found no increased incidence of birth defects in residents of an area contaminated with ß-naphthylamine, benzidine and benzene. Topham (1980, 1981) reported an increased incidence of sperm abnormalities in male mice injected intraperitoneally with benzidine at 50 mg/kg/day, but not at 100 or 250 mg/kg/day for 5 days. Wyrobek et al. (1981), however, found no significant increase in sperm abnormalities in mice similarly treated with ≤440 mg/kg/day for 5 days.

In mice exposed to benzidine dihydrochloride in the drinking water at 20-160 ppm for 33 months, there were dose-related decreases in body weight gain and survival at all levels; most deaths were caused by tumors (Littlefield et al., 1983, 1984). The incidence of hemosiderosis of the spleen was significantly increased in mice at ≥120 ppm. Other treatment-related effects included increased incidences of liver cell alterations in females at ≥20 ppm, bile duct hyperplasia in females at ≥40 ppm and in males at 160 ppm, megakaryocytosis of bone marrow in females at ≥60 ppm and in males at ≥120 ppm, bladder epithelial hyperplasia in males at 160 ppm, atrophy of the ovary in females at ≥60 ppm and brain vacuolization in females at ≥20 ppm and in males at ≥30 ppm.

Mice exposed to benzidine dihydrochloride in the drinking water at 50-500 ppm for up to 39 weeks had an increased incidence of mild periportal fibrosis, lymphocytic infiltration and brown pigmentation in the liver, hemosiderosis of the spleen and focal vacuolization of the urinary bladder epithelium at ≥50 ppm.

Dietary administration of benzidine dihydrochloride at 100-800 ppm for 6 weeks resulted in a dose-related loss of body weight of mice at ≥400 ppm (Rao et al., 1971; Vesselinovitch et al., 1975). Dose-related decreases in carcass, liver and kidney weights, dose-related increases in absolute spleen and thymus weights and dose-related increased severity of cloudy swelling of the liver, vacuolar degeneration of the renal tubules and hyperplasia of myeloid elements were also observed, but the doses were not specified (Rao et al., 1971).

Rats exposed to benzidine in the diet at 170 ppm died within 93-224 days and had fatty degeneration and regeneration of the bile ducts and fatty degeneration of the liver (Boyland et al., 1954). Rats exposed by inhalation to 10-20 mg/m³ benzidine, 4 hours/day, 5 days/week for up to 28 months had liver lesions suggestive of cirrhosis (Zabezhinskii, 1970).

The oral LD_{50} of benzidine is 309 mg/kg in rats and 110 mg/kg in mice (NIOSH, 1985).

The potency factor (B) of 234.13 $(mg/kg/day)^{-1}$ derived by U.S. EPA (1980a) from the human data of Zavon et al. (1973) is recommended as an interim upper limit value for estimating the increased lifetime risk of cancer for human exposure to benzidine. An F factor of 2361 $(mg/kg/day)^{-1}$ was derived from B. Benzidine is an IARC Group 1 and an EPA Group A human carcinogen with a HIGH hazard ranking under CERCLA. A chronic toxicity RQ of 100 was derived from the data for decreased survival of rats maintained on diets containing benzidine in the study by Boyland et al. (1954).

TABLE OF CONTENTS

		Page
1.	INTRODUCTION	1
1.1.	STRUCTURE AND CAS NUMBER	1
1.2.	PHYSICAL AND CHEMICAL PROPERTIES	1
1.3.	PRODUCTION DATA	2
1.4.	USE DATA	3
1.5.	SUMMARY	4
2.	ENVIRONMENTAL FATE AND TRANSPORT PROCESSES	5
2.1.	WATER	5
2.1.1.	Hydrolysis	5
2.1.2.	Oxidation	5
2.1.3.	Photolysis	5
2.1.4.	Microbial Degradation	6
2.1.5.	Volatilization	8
2.1.6.	Adsorption	8
2.1.7.	Bioaccumulation	8
2.1.8.	Persistence	9
2.2.	AIR	9
2.3.	SOIL	10
2.3.1.	Microbial Degradation	10
2.3.2.	Chemical Degradation	10
2.3.3.	Photolysis	10
2.3.4.	Adsorption	11
2.3.5.	Volatilization	11
2.3.6.	Persistence	11
2.4.	SUMMARY	12
3.	EXPOSURE	14
3.1.	WATER	14
3.2.	FOOD	15
3.3.	INHALATION	15
3.4.	DERMAL	17
3.5.	SUMMARY	17
4.	PHARMACOKINETCS	18
4.1.	ABSORPTION	18
4.2.	DISTRIBUTION	18
4.3.	METABOLISM	19
4.4.	EXCRETION	24
4.5.	SUMMARY	25

TABLE OF CONTENTS (cont.)

	Page
5. EFFECTS	21
5.1. CARCINOGENICITY	21
5.1.1. Animal Studies	21
5.1.2. Human Studies	48
5.2. MUTAGENICITY	54
5.3. TERATOGENICITY	56
5.4. OTHER REPRODUCTIVE EFFECTS	60
5.5. CHRONIC AND SUBCHRONIC TOXICITY	60
5.6. OTHER RELEVANT INFORMATION	64
5.7. SUMMARY	64
6. AQUATIC TOXICITY	69
6.1. ACUTE	69
6.2. CHRONIC	69
6.3. PLANTS	69
6.4. RESIDUES	71
6.5. OTHER RELEVANT INFORMATION	71
6.6. SUMMARY	72
7. EXISTING GUIDELINES AND STANDARDS	73
7.1. HUMAN	73
7.2. AQUATIC	73
8. RISK ASSESSMENT	74
9. REPORTABLE QUANTITIES	79
9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY	79
9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY	85
10. REFERENCES	89
APPENDIX: LITERATURE SEARCHED	115

LIST OF TABLES

No.	Title	Page
3-1	Monitoring Data for Benzidine in Water, Sediment and Tissues	16
4-1	Metabolites Formed by Biotransformation of Benzidine and Benzidine Derivitatives in Mammals	20
5-1	Incidence of Hepatocellular Carcinoma in Two Strains of Mice Given 100% Benzidine Dihydrochloride in Drinking Water for ~33 Months	28
5-2	Incidence of Harderian Gland Adenomas in Two Strains of Mice Given 100% Benzidine Dihydrochloride in Drinking Water for ~33 Months	30
5-3	Incidence of Uterine Angiomas in Two Strains of Female Mice Given 100% Benzidine Dihydrochloride in Drinking Water for ~33 Months	31
5-4	Incidence of Liver Tumors in a Drinking Water Study of Pure Benzidine Dihydrochloride in Mice	33
5-5	Incidence of Liver Tumors in Balb/c Mice Treated with Benzidine Dihydrochloride (Purity NR) in the Drinking Water for 39 Weeks	36
5-6	Incidence of Tumors in B6C3F1 Mice Treated Orally with Benzidine Dihydrochloride (Certified Grade)	38
5-7	Incidence of Liver Tumors in B6C3F1 Mice Fed Benzidine Dihydrochloride of Unspecified Purity During Various Developmental Periods	42
5-8	Incidence of Mammary Gland Tumors in Female Sprague-Dawley Rats Treated by Gavage with Benzidine (Pure) in Sesame Oil for 30 Days and Observed for 9 Months	44
5-9	Incidence of Liver Tumors in Syrian Golden Hamsters Given 1000 ppm Benzidine or the Dihydrochloride (Certified Grade) in the Diet for the Lifespan	46
5-10	Observed vs. Expected Numbers of Incident Cancers and SIR by Tumor Site	51
5-11	Mutagenicity Testing of Benzidine	57
6-1	Acute Toxicity of Benzidine to Aquatic Animals	70

LIST OF TABLES (cont.)

No.	Title	Page
9-1	Toxicity Summary for Benzidine.	80
9-2	Composite Scores for Benzidine.	82
9-3	Benzidine: Minimum Effective Dose (MED) and Reportable Quantity (RQ)	84
9-4	Derivation of Potency Factor (F) for Benzidine.	88

LIST OF ABBREVIATIONS

BCF	Bioconcentration factor
bw	Body weight
CHO	Chinese hamster ovary
CS	Composite score
DNA	Deoxyribonucleic acid
EC_{50}	Concentration effective to 50% of recipients (and all other subscripted concentration levels)
HPLC	High performance liquid chromatography
K_{oc}	Soil sorption coefficient
K_{ow}	Octanol/water partition coefficient
LC_{50}	Concentration lethal to 50% of recipients (and all other subscripted dose levels)
LD_{50}	Dose lethal to 50% of recipients
MED	Minimum effective dose
MC	Monohybrid cross
MTD	Maximum tolerated dose
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
ppb	Parts per billion
ppm	Parts per million
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value
SD	Standard deviation
SIR	Standardized incidence ratio
SMR	Standardized mortality ratio
SOCMA	Synthetic Organic Chemical Manufacturers Association

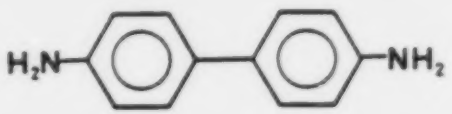
LIST OF ABBREVIATIONS (cont.)

TWA	Time-weighted average
tRNA	Transfer ribonucleic acid
UDS	Unscheduled DNA synthesis
UV	Ultraviolet

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

Benzidine is the common chemical name, but this compound is also known as (1,1'-biphenyl)-4-4'-diamine; 4,4'-diaminobiphenyl; 4,4'-bi-phenyl-diamine; 4,4'-bianiline; C.I. 37225; C.I. Azoic Diazo Component 112 and 4,4'-diphenylenediamine (IARC, 1982). The structure, molecular weight, empirical formula and CAS Registry number for benzidine are as follows:



Molecular weight: 184.24

Empirical formula: $C_{12}H_{12}N_2$

CAS Registry number: 92-87-5

1.2. PHYSICAL AND CHEMICAL PROPERTIES

Pure benzidine is a colorless, grayish-yellow or reddish-gray crystalline compound (Shriner et al., 1978) which darkens on exposure to light or air (Verschueren, 1983; Windholz, 1983). It is soluble in diethyl ether and ethanol (Shriner et al., 1978). Selected physical properties are listed below:

Melting point:	116-129°C (depending on isotropic forms) (IARC, 1982)
Boiling point:	400-401°C (Ferber, 1978)
Specific gravity (20/4°C):	1.250 (IARC, 1982)
Water solubility:	
at 12°C	400 ppm (Verschueren, 1983)
at 25°C	520 ppm (Shriner et al., 1978)
at 100°C	9400 ppm (Verschueren, 1983)
Vapor pressure (mm Hg) at unspecified temperature:	5×10^{-4} (calculated) (Mabey et al., 1981)

Log K_{ow}:	1.34 (recommended value) (Hansch and Leo, 1985)
Ionization Constants (20°C):	
pK_1	4.66 (Perrin, 1965)
pK_2	3.57 (Perrin, 1965)
Conversion Factor:	1 ppm = 0.133 mg/m³ (IARC, 1982)

Benzidine is both a primary aromatic amine and a diphenyl derivative, which undergoes chemical reactions characteristic of each of these classes of compounds (Shriner et al., 1978). It is sensitive to oxidation and can be readily acetylated, alkylated and diazotized. Diazotization reactions are commercially important in the use of benzidine to make dyes. Benzidine undergoes many characteristic color reactions with metal cations and oxidative anions (Ferber, 1978).

1.3. PRODUCTION DATA

Production of benzidine in the United States declined from 4 million pounds in 1948 to 1.5 million pounds in 1972 to 1.1 million pounds in 1974 (IARC, 1982). Production continued to decline steadily after 1974, and in 1976, all large-scale production of benzidine in the United States was discontinued (Ferber, 1978). Subsequent to 1976, two U.S. companies continued to manufacture benzidine dyes by a process in which the benzidine formed (as the dihydrochloride salt) was not isolated, but reacted directly to yield the dye. According to the U.S. EPA TSCA Production File (U.S. EPA, 1977), GAF Corp. produced 0.1-1.0 million pounds of benzidine dihydrochloride in 1977 for captive use. Columbia Organic Chemical (Columbia, SC) made <1000 pounds of benzidine in 1977 (U.S. EPA, 1977); Columbia primarily makes laboratory reagents.

From the list of dyes commercially derived from benzidine (Lurie, 1964), 11 were commercially produced in the United States in 1984 (USITC, 1985); 10 of these dyes were made by Fabricolor Manufacturing Corp. of Patterson, NJ and one was made by Crompton & Knowles Corp. of Reading, PA.

The last year in which benzidine was reported to be imported through principal United States Customs districts was 1980, when 8990 pounds were imported (USITC, 1981).

Benzidine is commercially manufactured by treating an alkaline solution of nitrobenzene with a mild reducing agent, such as zinc dust, to form hydrazobenzene (Shriner et al., 1978). The hydrazobenzene is then reacted with a mineral acid to yield benzidine; the use of hydrochloric acid results in the formation of benzidine dihydrochloride (IARC, 1982), which is used for subsequent production of various dyes (Ferber, 1978). Historically, most of the benzidine produced in the United States was for on-site captive use (Ferber, 1978).

1.4. USE DATA

The dominant commercial use of benzidine has remained the production of direct azo dyes. The amount consumed in all other benzidine applications is insignificant by comparison (IARC, 1982). As many as 254 dyes and pigments can be synthesized from benzidine (IARC, 1982); however, only 11 of these dyes were commercially produced in the United States in 1984 (see Section 1.3.).

The following minor uses of benzidine or its salts have been reported (IARC, 1982; Shriner et al., 1978): blood detection in clinic and criminal investigations; hardener in the rubber industry; hardener in adhesives and plastics; detection of H_2O_2 in milk; security printing (reacts with ink

erasers); detection of inorganic ions and compounds; quantitative determination of nicotine; spray reagent for sugars; metal analysis; pesticide chromatography; detection of bacterial cytochromes; detergent determination; synthesis of intermediates; detection of chlorine or pyridine in drinking water and detection of cresols. It is not known to what extent, if any, benzidine is still used in any of these applications (IARC, 1982).

1.5. SUMMARY

Benzidine is a crystalline compound that had been important in commerce because of its usefulness as an intermediate in the manufacturing of azo dyes. All large-scale production of benzidine as an endproduct was terminated in the United States in 1976; however, its production as a nonisolated intermediate (as the dihydrochloride salt) has continued by U.S. dye manufacturers (Ferber, 1978; IARC, 1982). From the list of dyes commercially derived from benzidine (Lurie, 1964), a total of 11 dyes were commercially produced in the United States in 1984 by two dye manufacturers (USITC, 1985). In comparison to the use of benzidine as an azo dye intermediate, the amount of benzidine consumed in all other applications is insignificant (IARC, 1982). Most of the reported minor uses of benzidine (IARC, 1982; Shriner et al., 1978) involve its use as an analytical detection agent.

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

2.1. WATER

2.1.1. Hydrolysis. Benzidine does not contain hydrolyzable functional groups in its chemical structure (Mabey et al., 1981). Therefore, the hydrolysis of benzidine in water is not considered important (Mabey et al., 1981; Callahan et al., 1979; U.S. EPA, 1981).

2.1.2. Oxidation. Benzidine is rapidly oxidized by metal cations (U.S. EPA, 1981) and other cations that are found in environmental waters (Callahan et al., 1979). Oxidation by cations of natural waters may be the fastest environmental fate process for benzidine in water (Callahan et al., 1979); however, sufficient rate data are not available to predict the kinetics of cation oxidation in the ambient environment.

Mabey et al. (1981) theoretically calculated the rate constant for the reaction between benzidine and peroxy radical ($RO_2\cdot$) in aqueous solution at 25°C to be 1.1×10^{8} M^{-1} hr^{-1}. Peroxy radicals are formed in natural waters by photooxidation processes initiated by sunlight; the concentration of $RO_2\cdot$ in sunlit natural water is $\sim 10^{-9}$ M (Mabey et al., 1981). Therefore, the half-life for the benzidine-$RO_2\cdot$ reaction can be calculated to be 6.3 hours.

In addition to oxidation by cations and peroxy radical, benzidine can be oxidized in water by direct reaction with oxygen (U.S. EPA, 1981; Callahan et al., 1979).

2.1.3. Photolysis. The absorption spectrum for an aqueous benzidine solution (Lahav and Raziel, 1971) shows significant absorption in the range from 290-330 nm, indicating a potential for direct photolysis. Banerjee et al. (1978) measured the disappearance quantum yield for benzidine in aqueous solution to be 0.012 at 300 nm and found minimal dependence of quantum yield

upon wavelength. This quantum yield value suggests that direct photolysis of benzidine in the aquatic environment will occur, but the reaction may not be competitive with oxidation.

While the direct photolysis of benzidine in natural bodies of water may not be rapid, the indirect photolysis or photosensitized photolysis may be more significant. Benzidine is readily adsorbed by clay particles and organic matter that are present in natural waters (Lahav and Raziel, 1971; Furukawa and Brindley, 1973; Callahan et al., 1979; Zierath et al., 1980). The humic acids and fulvic acids present in natural water absorb sunlight and can initiate the photolysis of compounds that are adsorbed to them by energy transfer (Mill and Mabey, 1985). In addition, benzidine undergoes a chemical oxidation when adsorbed to clay (Lahav and Raziel, 1971); the absorption spectrum for benzidine added to bentonite clay in aqueous solution shows extremely strong absorption of wavelengths between 290 and 700 nm (Lahav and Raziel, 1971) and may therefore photodegrade significantly in natural waters. Callahan et al. (1979) noted that the benzidine radical cation that is formed by the oxidation of benzidine by montmorillonite may undergo photodegradation since it absorbs light strongly.

Howard and Saxena (1976) reported and commented on the results of various aqueous degradation tests using benzidine conducted by the SOCMA Benzidine Task Force. The experimental design of the photolysis tests, however, was insufficient to determine the importance of photolysis in water (Howard and Saxena, 1976).

2.1.4. **Microbial Degradation.** The SOCMA Benzidine Task Force examined the degradation of benzidine under conditions that may be encountered in a sewage treatment plant (Howard and Saxena, 1976). Systems using both aniline-acclimated sludge and unacclimated sludge were shown to remove as

much as 95% of initial benzidine; however, the degradation of the benzidine was a combination of oxidation and biodegradation. Results of Warburg tests suggested that benzidine is not easily biodegraded (Howard and Saxena, 1976).

Lutin et al. (1965) studied the biodegradation of benzidine in a Warburg apparatus with unacclimated activated sludge at a benzidine concentration of 500 ppm. Oxygen uptake was found to be less than the control, suggesting that benzidine was not biodegraded.

Tabak and Barth (1978), using a flow reactor designed to simulate sludge-treatment operations, found that 6 weeks were required for acclimation of activated sludge exposed to 5 ppm of benzidine; at concentrations $\geq$10 ppm, acclimation was not achieved. In a similar activated sludge degradation study, Freitag et al. (1984) found relatively little biodegradation of 0.05 ppm benzidine after 5 days of incubation. The U.S. EPA (1981) reported 0% removal of benzidine at a concentration of 0.004 ppm in a full-scale wastewater treatment plant.

Baird et al. (1977) used Warburg bioassay techniques to demonstrate that, although benzidine showed some inhibiting effects on the oxygen uptake of microorganisms in two commercial activated sludges, significant biodegradation of benzidine still occurred, particularly at low benzidine concentrations. Fochtman (1981) also found benzidine to exhibit some biological degradation by biological treatment practices.

The above data pertain to activated sludge biodegradation of benzidine. The ability of benzidine to be biodegraded in natural waters is not known. Based on an estimated biotransformation rate constant of 10^{-10} mℓ cell^{-1} hour^{-1} (Mabey et al., 1981) and the concentration of microorganism of 10^{6} cell mℓ (Odunlan and Odeyemi, 1984), biodegradation does not appear to be a significant process.

2.1.5. **Volatilization.** Pertinent data regarding volatilization could not be located in the available literature as cited in the Appendix. Mabey et al. (1981) calculated the Henry's Law constant for benzidine to be 3×10^{-7} atm-m³/mol at 12°C based on the water solubility of benzidine at 12°C (400 ppm) and calculated vapor pressure data. This value of Henry's constant indicates that volatilization from natural bodies of water is not important (Lyman et al., 1982). The U.S. EPA (1981) also reported that volatilization was not significant.

2.1.6. **Adsorption.** Benzidine is strongly adsorbed by clay particles (Callahan et al., 1979; Furukawa and Brindley, 1973; Lahav and Raziel, 1971; U.S. EPA, 1981) and by sediment (Zierath et al., 1980). Therefore, adsorption of benzidine in water is potentially important.

2.1.7. **Bioaccumulation.** Lu et al. (1977) examined the uptake of benzidine by aquatic organisms in a closed model ecosystem for 3 days. The following BCFs were determined:

Gambusia affinis (fish)	110
Culex pipiens quinquefasciatus (mosquito, larva)	1180
Physa sp. (snail)	370
Oedogonium cardiacum (alga)	160

Degradation of the benzidine in the ecosystem proceeded through N-methylation and acetylation of the amino groups.

Freitag et al. (1984) measured the bioconcentration of benzidine in fish (Golden orfe) during a 3-day exposure to 0.05 ppm; a BCF value of 83 was determined. Exposure of the algae, Chorella fusca, for 1 day resulted in a BCF of 850. Shriner et al. (1978) reported a BCF value of 44 in bluegill fish.

2.1.8. Persistence. The SOCMA Benzidine Task Force conducted die-away tests with benzidine in chlorinated, aerated and nonaerated lake water obtained near Buffalo, NY (Howard and Saxena, 1976). The degradation rate constant determined (~0.175/hour; half-life of 4 hours) did not differ significantly under the different conditions. The results indicated that benzidine was rapidly degraded in the lake water; however, the pathway of degradation was not clear. Photolysis and microbial degradation did not appear to be important (Howard and Saxena, 1976), but it was not certain that oxidative processes were responsible for the entire degradation.

2.2. AIR

Experimental data regarding the environmental fate processes for benzidine in air were not located. Shriner et al. (1978) reported an estimated half-life of 1 day for the reaction of benzidine with OH radicals and ozone in air. The structurally similar compounds aniline and biphenyl have the following rate constants for the reaction with OH radical at 23°C (Atkinson, 1985):

aniline	119×10^{-12} cm³/molecule-sec
biphenyl	5.8×10^{-12} cm³/molecule-sec

Assuming an average atmospheric OH• concentration of 10^6 molecules/cm³, the half-lives for aniline and biphenyl can be calculated to be 1.6 hours and 1.4 days, respectively. Therefore, an average half-life of 1 day for benzidine seems reasonable.

Benzidine absorbs sunlight in the region 290-330 nm (Sadtler, n.d.); therefore, direct photolysis in air is potentially possible. The quantum yield for aqueous benzidine of 0.012 at 300 nm and pH 7.0 (Banerjee et al., 1978) indicates that photolysis in air may not be as rapid as the reaction with OH• radicals. Benzidine darkens on exposure to light (Windholz, 1983), suggesting a photodegradative process.

Benzidine is susceptible to oxidation by oxygen (Callahan et al., 1979; U.S. EPA, 1981); this process may also be important in the removal of benzidine from air.

Data regarding the physical removal of benzidine from air could not be located in the available literature as cited in the Appendix.

2.3. SOIL

2.3.1. Microbial Degradation. Benzidine was found to be moderately susceptible to biodegradation after 7 days of incubation in an artificial compost mixture consisting of shredded newspaper, manure, sludge, sawdust, peat moss, soil, powdered milk and fertilizer (Snell Environmental Group, Inc., 1982). Yoshida et al. (1973) found benzidine to be stable even after 9 weeks of incubation in a soil bacteria degradation study. Graveel (1984) used sterile and nonsterile soils to demonstrate that benzidine can be significantly biodegraded in soil.

2.3.2. Chemical Degradation. When benzidine is brought into contact with montmorillonite clays, an intense blue complex is formed because of the oxidation of benzidine to radical cations (Callahan et al., 1979; Lahav and Raziel, 1971). This reversible oxidation is stabilized by adsorption to the clay (Zierath et al., 1980). In the absence of clay or other suitable sorbents, benzidine is irreversibly oxidized to a brown degradation product (Zierath et al., 1980).

2.3.3. Photolysis. Kotzias et al. (1981) irradiated benzidine adsorbed to silica gel at a wavelength >290 nm for 3 days. After 3 days, 40.8% of the initially adsorbed benzidine had been photomineralized to CO_2. This suggests that benzidine exposed to sunlight on soil surfaces is susceptible to photolysis. Lahav and Raziel (1971) have shown that the benzidine-clay complex formed by oxidation of the benzidine absorbs sunlight radiation

much more intensely than benzidine itself; therefore, this complex is likely to photodegradate when exposed to sunlight.

2.3.4. **Adsorption.** Zierath et al. (1980) examined the sorption of benzidine by 14 different types of soils and sediments. The sorption experiments were conducted with ^{14}C-labeled benzidine and stainless steel equilibration tubes. Based on the Freundlich constants, which were experimentally determined for the sorption by each soil and sediment and the organic content of each soil and sediment, the K_{oc} values were calculated to range from 264-82,110 for the 14 soil and sediment types. The K_{oc} value is typically in the thousands, which is indicative of low soil mobility. The sorption was found to show pH dependence; the effect of pH was explained by noting that benzidine can exist in solution as both an ionized (cationic) species and a neutral species with pH controlling this distribution. Both species are subject to significant sorption.

Sorption of benzidine to clay minerals is so rapid that it is difficult to measure the kinetics of the process (Callahan et al., 1979). This adsorption results in oxidation to the blue complex.

These data indicate that benzidine is not expected to leach significantly in most types of soil.

2.3.5. **Volatilization.** The low vapor pressure ($\sim 10^{-4}$ mm Hg at ambient temperatures) and high soil adsorption of benzidine indicate that volatilization from soil is not expected to occur.

2.3.6. **Persistence.** Lu et al. (1977) examined the rate of disappearance of benzidine from a spiked silty clay loam soil. The tests were conducted in Petri dishes containing 10 g of soil. The amount of initially added benzidine remaining in the Petri dishes after 1 week was 29.26% and after 4 weeks was 20.67%.

2.4. SUMMARY

Oxidation is probably the most important fate process for benzidine in aquatic environments. Benzidine is rapidly oxidized by metal cations (U.S. EPA, 1981) and other cations found in natural waters (Callahan et al., 1979). In addition, oxidation by peroxy radicals in sunlit waters occurs rapidly (Mabey et al., 1981), with a calculated half-life of 6.3 hours. Benzidine can also be oxidized in water by direct reaction with oxygen (U.S. EPA, 1981; Callahan et al., 1979). Photosensitized photolysis of benzidine or photolysis of benzidine-oxidation complexes in water may have some importance. Biodegradation in natural water is not expected to be a significant process. Benzidine is strongly adsorbed by clay particles (Callahan et al., 1979; Furukawa and Brindley, 1973; Lahav and Raziel, 1971; U.S. EPA, 1981) and by sediment (Zierath et al., 1960) suggesting importance of adsorption in aquatic environments. Hydrolysis and volatilization from water are not environmentally important (Mabey et al., 1981; Callahan et al., 1979; U.S. EPA, 1981). Experimentally determined BCFs of 44, 83 and 110 have been reported in fish (Shriner et al., 1978; Freitag et al., 1984; Lu et al., 1977). A persistence study conducted with a single lake water found benzidine to have a half-life of 4 hours (Howard and Saxena, 1976).

In the atmosphere, benzidine is probably removed rapidly by reaction with OH radicals. Based on reaction kinetics (Atkinson, 1985) of structurally similar compounds (aniline and biphenyl), the half-life for the benzidine reaction with OH radicals is ~1 day. Photolysis and direct oxidation with oxygen may also contribute to the removal of benzidine from air.

Benzidine is rapidly oxidized by clay to a blue complex (Callahan et al., 1979; Lahav and Raziel, 1971). This reversible oxidation is stabilized by adsorption to the clay (Zierath et al., 1980). In the absence of clay or

other suitable sorbents, benzidine is irreversibly oxidized to a brown degradation product (Zierath et al., 1980). The UV absorption spectrum for benzidine and the benzidine-clay oxidation complexes (Lahav and Raziel, 1971) and the significant photolysis of benzidine adsorbed to silica gel (Kotzias et al., 1981) suggest that photolysis on sunlit soil surfaces is likely to occur. Based on sorption coefficients of 14 different soils and sediments (Zierath et al., 1980), benzidine is not expected to leach significantly in most types of soil.

3. EXPOSURE

Benzidine has not been reported to occur naturally in the environment (IARC, 1982).

3.1. WATER

Takemura et al. (1965) detected benzidine in the Sumida River in Japan at concentrations ranging from 0.082-0.257 ppm. The river water samples were collected downstream from dye factories. The authors suggested that the presence of benzidine in the river was not necessarily due to direct discharges from the factories, but may have resulted from the reduction of azo-dyes in the waste by H_2S or SO_2 in the river. Yoshida et al. (1973) demonstrated that benzidine is formed by the degradation of a direct black azo dye. Boeniger et al. (1981) have shown that both domestic and imported dyes based on benzidine contain residual amounts of benzidine; therefore, wastewater discharges from plants producing or using benzidine-derived dyes may contain some of this residual benzidine.

Shackelford and Keith (1976) reported the detection of benzidine in the Rhine River and in the effluent from a raw sewage treatment facility in the United States. Hauser and Bromberg (1982) detected benzidine in water-soil-sediment samples taken from the Love Canal in New York State. The U.S. EPA (1981) reported the identification of benzidine in the raw wastewater from the nonferrous metals manufacturing industry (6.0 ppb max.) and in the raw wastewater from a foundry (10 ppb max.). Effluents from textile and leather factories using benzidine-derived dyes were found to contain average benzidine concentrations of 0.25-3.5 ppb (IARC, 1982). Water and sediment samples collected downstream on the Buffalo River from a plant using benzidine and its dyes were found to contain no detectable benzidine residues (Howard and Saxena, 1976).

Benzidine exposure to natural waters can also result from waste product emissions from coal conversion plants (Hassett et al., 1980). Banerjee et al. (1978) identified benzidine as a photodegradation product of 3,3'-dichlorobenzidine; this suggests that benzidine may be formed in natural waters by decomposition of compounds other than dyes.

The gross analysis of data for benzidine available from the U.S. EPA STORET Data Base is listed in Table 3-1. Staples et al. (1985) reported that 1.1% of reporting stations in STORET for idustrial effluents had positive levels of benzidine while only 0.1% of ambient water stations had positive benzidine level.

3.2. FOOD

Data pertaining to the detection of benzidine in food were not located. It is possible that food may become contaminated under poor industrial hygienic conditions, such as storage in dyed containers (U.S. EPA, 1980a), but this route of exposure seems remote.

3.3. INHALATION

Monitoring data concerning the detection of benzidine in the ambient atmosphere could not be located in the available literature as cited in the Appendix. Airborne concentrations of benzidine ranging from 0.007-17.6 mg/m^3 have been found at various process locations of a benzidine manufacturing plant (IARC, 1982); however, since benzidine is no longer industrially produced in the United States, these occupational concentrations are not relevant. Data on the occupational levels of benzidine at user sites could not be located in the available literature as cited in the Appendix.

TABLE 3-1

Monitoring Data for Benzidine in Water, Sediment and Tissues[a]

Concentration	Water[b] (µg/ℓ)	Sediment[c] (µg/kg dry weight)	Aquatic Organism Tissue[d] (mg/kg wet weight)
Maximum	405,324	770,000	140
Minimum	ND	ND	ND
Mean	206	3,946	5

[a]Source: U.S. EPA, 1986

[b]5630 reporting stations

[c]855 reporting stations

[d]563 reporting stations

ND = Not detected

3.4. DERMAL

The greatest risk for dermal exposure to benzidine is likely to result from occupational situations at plants using and producing benzidine-derived dyes. In a Polish factory manufacturing benzidine, workers were found to have average benzidine concentrations on the skin of 0.26-0.80 $\mu g/cm^2$ (Bolanowska et al., 1972).

3.5. SUMMARY

Benzidine has been detected in the effluents from factories using benzidine-derived dyes (IARC, 1982), in river water downstream from dye factories (Takemura et al., 1965) and in other industrial effluents (U.S. EPA, 1981). Although benzidine-derived dyes contain residual amounts of benzidine (Boeniger et al., 1981), the presence of benzidine in certain waters receiving dye wastes may be the result of the reduction of the dye to yield benzidine as a decompostion product (Takemura et al., 1965); the degradation of a benzidine dye has been shown to yield benzidine (Yoshida et al., 1973). Detection of benzidine in food or in the ambient atmosphere has not been reported.

4. PHARMACOKINETICS

4.1. ABSORPTION

Little information is available concerning the absorption of benzidine. The absorption of benzidine from the gastrointestinal tract can be inferred from the systemic toxicity and carcinogenicity observed in laboratory animals following oral dosing (Chapter 5). After oral, inhalation or dermal exposure of dogs to benzidine, Ghetti (1960) observed varying concentrations of benzidine or its metabolites in the urine. Benzidine is readily absorbed through the skin (Meigs et al., 1951; Barsotti and Vigliani, 1952). As reported in an abstract of a Polish study by Krajewska et al. (1980), the mean annual dose of benzidine absorbed by workers was 0.065 mg. Only 10% of the dose was attributable to inhalation exposure whereas, 90% of the dose was absorbed through the skin.

4.2. DISTRIBUTION

Kellner et al. (1973) administered ^{14}C-benzidine intravenously to rats, monkeys and dogs at a dose of 0.2 mg/kg. The label was rapidly and widely distributed within 1 hour. Low residue levels were still detected 7 and 14 days after treatment. The blood elimination curves were multiphasic with the half-lives for the terminal (Phase IV) phase of 88 hours for dogs and 65 hours for rats. Lynn et al. (1984) reported that the major portion of radioactivity from an intravenous dose of 2.5 mg/kg of ^{14}C-labeled benzidine was accumulated in the intestinal tract of rats, as indicated by the excretion of 71% of the label in the bile. The liver contained the highest concentration of label as compared with other tissues. Baker and Deighton (1954) intraperitoneally injected rats with 100 mg/kg of benzidine. Four hours postinjection, high concentrations were observed in the stomach and its contents as well as in the small intestine. Twelve hours after

injection benzidine was found in the contents of the small intestine. At both 4 and 12 hours the amine content of erythrocytes was low. After 12 hours, high concentrations of conjugated material (indicative of metabolites) were observed in the tissues and urine. Benzidine concentrations in the liver were consistently high for the entire 12-hour period. Engelbertz and Babel (1956) reported that ^{14}C-labeled benzidine administered intravenously to dogs was distributed to the liver (12%), bile (10%), intestine (15%) and urine (31%) 4 hours after dosing.

4.3. METABOLISM

The metabolism of benzidine was reviewed by U.S. EPA (1980a) and Haley (1982). Because of the abundance of information available for benzidine, this review will concentrate on relatively recent data.

U.S. EPA (1980a) summarized the following information concerning the metabolism of benzidine in humans. Englebertz and Babel (1953) found that a single oral dose of 100 mg benzidine was excreted in urine as free benzidine and as its mono- and di-acetylated conversion products. Troll et al. (1963) observed that humans ingesting 200 mg of benzidine excreted N-hydroxyacetylamino benzidine and free benzidine in the urine. Plant workers exposed to an unknown quantity of benzidine had free benzidine, 3-hydroxybenzidine and the mono- and di-acetylated derivatives of benzidine in urine (Sciarini and Meigs, 1961a). Meigs et al. (1954) suggested that an 8-hour exposure to an air concentration of 0.018 mg/m^3 benzidine resulted in urinary excretion of 0.026 mg/ℓ of the diamines. U.S. EPA (1980a) also indicated benzidine metabolites vary considerably with species (Table 4-1). These differences are attributable to the presence or absence of specific enzyme pathways.

TABLE 4-1

Metabolites Formed by Biotransformation of Benzidine and Benzidine Derivatives in Mammals*

Compound	Species	Metabolites	Reference
Benzidine	human	acetyl N-hydroxy compound	Troll et al., 1963
	human	N-hydroxy acetylamino-benzidine	Haley, 1975
	human	monoacetylbenzidine and diacetylbenzidine	Haley, 1975
	human	3-hydroxybenzidine	Haley, 1975
	human	3,3'-dihydroxybenzidine	Haley, 1975
	monkey	monoacetylbenzidine	Rinde and Troll, 1975
	dog	3-hydroxybenzidine and glucuronide	Troll and Nelson, 1958
	dog	3-hydroxybenzidine hydrogen sulfate	Sciarini and Meigs, 1958
	dog	3-hydroxybenzidine	Bradshaw and Clayson, 1955
	dog	4,4'diamino-3-diphenyl hydrogen sulfate	Clayson et al., 1959
	dog	4-amino-4-hydroxybiphenyl	Haley, 1975
	dog	monoacetylbenzidine and diacetylbenzidine	Haley, 1975
	guinea pig	4'-acetamido-4-amino-diphenyl	Clayson et al., 1959
	guinea pig	4'-acetamido-4-amino-3-diphenyl hydrogen sulfate	Clayson et al., 1959
	guinea pig	4'-amino-4-diphenyl sulfamic acid	Clayson et al., 1959
	guinea pig	N-glucuronides	Clayson et al., 1959

TABLE 4-1 (cont.)

Compound	Species	Metabolites	Reference
Benzidine	guinea pig	4'-acetamido-4-diphenyl sulfamic acid	Clayson et al., 1959
	rabbit	3-hydroxybenzidine sulfate and glucuronide	Troll and Nelson, 1958
	rabbit	4'-acetamido-4-amino-diphenyl	Clayson et al., 1959
	rabbit	3-hydroxybenzidine	Clayson et al., 1959
	rabbit	4'-acetamido-4-amino-3-diphenylyl hydrogen sulfate	Clayson et al., 1959
	rabbit	4'-amino-4-diphenylyl sulfamic acid	Clayson et al., 1959
	rabbit	4'-acetamido-4-diphenylyl sulfamic acid	Clayson et al., 1959
	rabbit	N-glucuronides	Clayson et al., 1959
	rat	3,3'-dihydroxybenzidine	Haley, 1975
	rat	N-glucuronides	Elson et al., 1958; Clayson et al., 1959
	rat	4'-acetamido-4-amino-diphenyl	Clayson et al., 1959
	rat	3-hydroxybenzidine	Clayson et al., 1959
	rat	4,4'-diamino-3-diphenylyl hydrogen sulfate	Clayson et al., 1959
	rat	4'-acetamido-4-amino-3-diphenylyl hydrogen sulfate	Clayson et al., 1959
	rat	4'-amino-4-diphenylyl sulfamic acid	Clayson et al., 1959
	rat	4'-acetamido-4-diphenylyl sulfamic acid	Clayson et al., 1959

TABLE 4-1 (cont.)

Compound	Species	Metabolites	Reference
Benzidine	mouse	monoacetylbenzidine and diacetylbenzidine	Sciarini and Meigs, 1961a
	mouse	monoacetylated 3-hydroxy-benzidine glucuronide and/or ethereal sulfate	Sciarini and Meigs, 1961a
	mouse	N-hydrogen sulfate and/or glucuronide	Sciarini and Meigs, 1961a
	mouse	3-hydroxybenzidine glucuronide	Sciarini and Meigs, 1961a
	mouse	4'-acetamido-4-amino-diphenyl	Clayson et al., 1959
	mouse	4,4'-diamino-3-diphenyl hydrogen sulfate	Clayson et al., 1959
	mouse	4'-acetamido-4-amino-3-diphenylyl hydrogen sulfate	Clayson et al., 1959
	mouse	N-glucuronides	Clayson et al., 1959
3,3'-Dimethyl-benzidine (orthotolidine)	human	diacetyl-o-tolidine	Dieteren, 1966
	human	5-hydroxy-o-tolidine	Dieteren, 1966
	human	monoacetyl-o-tolidine	Dieteren, 1966
	dog	5-ethereal sulfate of o-tolidine	Sciarini and Meigs, 1961b
3,3'-Dimethoxy-benzidine (dianisidine)	dog	unidentified diamine metabolite	Sciarini and Meigs, 1961b
3-Methoxyben-zidine (mono-substituted dianisidine)	rat	4-amino-4'-acetamido-3-methoxybiphenyl	Laham, 1971

*Source: U.S. EPA, 1980a

For example, dogs do not readily acetylate benzidine; whereas, humans readily acetylate this compound. Lynn et al. (1984) identified the urinary metabolites in rats treated orally with 0.5-50 mg/kg. The major metabolites separated and identified by HPLC were as follows: N-acetyl benzidine; N,N'-diacetyl benzidine; benzidine-N-glucuronide; N-acetyl benzidine-glucuronide; N-hydroxy-N,N'-diacetyl benzidine-glucuronide; 3-hydroxy-N,N'-diacetyl benzidine-glucuronide; and a glutathione conjugate of N,N'-diacetyl benzidine. The major urinary or biliary metabolites at doses of 0.5-5 mg/kg were 3-hydroxy-N,N'-diacetyl benzidine-glucuronide, the glutathione conjugate and N,N'-diacetyl benzidine. At the high dose (50 mg/kg), N-hydroxy-N,N'-di- acetyl benzidine-glucuronide was the major urinary and biliary metabolite.

Several recent in vitro metabolism studies were located. Morton et al. (1979) observed that the liver cytosol from rats, mice, hamsters and guinea pigs metabolized benzidene to N-acetylbenzidine and N-N'-diacetylbenzidine. Hepatic microsomal preparations converted synthetic N,N'-diacetylbenzidine to N-hydroxy-N-N'diacetylbenzidine and 3-hydroxy-N-N'diacetylbenzidine in vitro by a NADPH-dependent reaction. Synthetic N-hydroxy-N-acetyl-N'-[1-^{14}C]-acetylbenzidine binds to tRNA in vitro when catalyzed by the liver cytosol. Thus, rodent livers contain the necessary enzymes for metabolizing benzidine to a product that binds to nucleic acids. Rat and mouse hepatic sulfotransferases catalyze the esterification of N-hydroxy-N,-N'-diacetyl benzidine to an electrophilic reactant (Morton et al., 1980). Zenser et al. (1979, 1980) found that ^{14}C-labeled benzidine was converted to products that covalently bind to exogenous tRNA and DNA in microsomal preparations from the inner renal medulla of male New Zealand rabbits. The reaction appeared to be mediated by prostaglandin endoperoxide synthetase. Prostaglandin endoperoxide synthetase in the presence of arachidonic acid

metabolized benzidine to mutagenic products (Robertson et al., 1983). Martin and Ekers (1980) intraperitoneally injected male Wistar rats with 32 mg/kg bw ^{3}H-benzidine. The label was incorporated into rat liver RNA, DNA and protein. The DNA associated label was stable for 4 weeks.

4.4. EXCRETION

Rats given an intravenous dose of 0.2 mg/kg bw ^{14}C-benzidine excreted ~80% of the administered radioactivity in the feces within 7 days of treatment (Kellner et al., 1973). Monkeys and dogs given 0.2 mg/kg bw ^{14}C-benzidine excreted ~30% of the radiolabel in the feces. Dogs, monkeys and rats excreted ~67, 50 and 17% of the radioactivity in urine. Kellner et al. (1973) reported that the urine and feces elimination curves were multiphasic. Half-lives for the terminal phases (Phase IV) were 38 hours for urine and 41 hours for feces in rats and 66 hours for urine and 29 hours for feces in dogs. Elimination by monkeys was faster, but half-lives or elimination curves were not provided for the monkey. Lynn et al. (1984) administered ^{14}C-labeled benzidine either orally (5.0 mg/kg) or intravenously (2.5 mg/kg) to rats. Three days after administration, 90% of the label was excreted (25% in the urine and 65% in the feces); 7% of the label was recovered in the carcass. The high amounts of fecal excretion and the accumulation of radioactivity in the gastrointestinal tract (see Section 4.2.) after parenteral dosing with ^{14}C-benzidine indicates a high degree of biliary excretion. The long residence time in the liver (Kellner et al., 1973) also suggests enterohepatic recirculation. In workers manufacturing benzidine, Sciarini and Meigs (1961a) reported urinary levels of benzidine and its metabolites of 0.23-0.67 mg/ℓ with values up to 21.8 mg/ℓ. An average of 4-6% was excreted as free benzidine, 2-5% as the monoacetylbenzidine and 5-10% as the diacetylbenzidine. The remainder was excreted as conjugates.

4.5. SUMMARY

Benzidine is absorbed through the skin (Meigs et al., 1951; Barsotti and Vigliani, 1952), the gastrointestinal tract and the lung (Ghetti, 1960).

Radioactivity was rapidly and widely distributed to tissues of dogs, monkeys and rats within 1 hour of intravenous dosing with ^{14}C-benzidine (Kellner et al., 1973). Blood elimination curves were multiphasic, and low residue levels were still detected 7 and 14 days after dosing. Following parenteral administration of ^{14}C-benzidine to rats and dogs, radiolabel was found in bile, and was accumulated in the gastrointestinal tract (Kellner et al., 1973; Lynn et al., 1984; Engelbertz and Babel, 1956). Benzidine metabolites vary considerably among species (U.S. EPA, 1980a). Benzidine is acetylated and hydroxylated in humans (Englebertz and Babel, 1953; Troll et al., 1963; Sciarini and Meigs, 1961a). Dogs do not acetylate benzidine appreciably, while rats readily acetylate and conjugate the compound (Lynn et al., 1984). The metabolites produced in rats are dependent on dose (Lynn et al., 1984). Several recent in vitro metabolism studies were located. Morton et al. (1979, 1980) found that the liver cytosol from rats, mice, hamsters and guinea pigs acetylated benzidine. These investigators also noted rodent livers have the necessary enzymes to convert benzidine to a product that binds nucleic acids. Zenser et al. (1979, 1980) obtained the same results for microsomes from the inner renal medulla of rabbits with mediation by prostaglandin endoperoxide synthetase. Martin and Ekers (1980) observed that rats given intraperitoneal injections of labeled benzidine incorporated label into liver nucleic acids and proteins.

Rats predominantly excrete benzidine and its metabolites in the feces (Kellner et al., 1973; Lynn et al., 1984), while dogs and monkeys excrete benzidine and its metabolites primarily in urine (Kellner et al., 1973),

although fecal excretion is also appreciable. Elimination curves for urine and feces of dogs and rats are multiphasic (Kellner et al., 1973), which (along with the findings of radioactivity in bile and the gastrointestinal tract after parenteral dosing with ^{14}C-benzidine, the long residence time in the liver, tissues and carcass, and the high degree of conjugated metabolites and fecal excretion) indicates that biliary excretion and enterohepatic recirculation occur extensively.

5. EFFECTS

5.1. CARCINOGENICITY

5.1.1. Animal Studies. Littlefield et al. (1983, 1984) studied the carcinogenicity of benzidine in two strains of male and female mice (Table 5-1). Groups of 72-120 male mice of each strain were exposed to 0, 30, 40, 60, 80, 120 or 160 ppm, while groups of 72-120 female mice of each strain were exposed to 0, 20, 30, 40, 60, 80 or 120 ppm of benzidine dihydrochloride in drinking water for ~33 months. Both strains of mice had dose-related decreased body weight gain and survival, and dose-related increased incidences of liver neoplasms. Each of these endpoints varied according to sex and strain; in general, females were more susceptible than males, and the F_1 strain was more susceptible than the MC strain to the effects of benzidine dihydrochloride. The incidence of hepatocellular carcinomas increased with increased dose (see Table 5-1), with the exception of MC males whose hepatocellular carcinoma incidence reached a maximum of 37/71 at 120 ppm and diminished to 32/71 at 160 ppm. There was a significant dose-related trend in time elapsed before tumor formation. The estimated mean time-to-tumor for F_1 females (which were representative of trends for both strains and sexes) was 464 days at 120 ppm as compared with 1023 days for controls. Water consumption data were also reported. Other tumor types (reticulum cell sarcomas, lymphomas, mammary, lung, etc.) were also listed as causes of death, but no incidence data were reported. Littlefield et al. (1983) indicated a dose-response relationship with respect to time in the prevalence of lung tumors and deaths attributable to reticulum cell sarcomas; these relationships were independent of the presence of liver tumors. Littlefield et al. (1983) also reported that the increased incidences of adenoma of the Harderian gland (Table 5-2) and uterine angioma (Table 5-3) were also treatment-related.

TABLE 5-1

Incidence of Hepatocellular Carcinoma in Two Strains of Mice Given 100% Benzidine Dihydrochloride in Drinking Water for ~33 Months[a]

Strain[b]	Sex	Concentration (ppm)	Corresponding Dose (mg/kg/day)[c]	Tumor Incidence (%)
F_1	M	0	0	14/125 (11)
F_1	M	30	3.7	24/119 (20)
F_1	M	40	4.7	30/96 (31)
F_1	M	60	7.5	23/71 (32)
F_1	M	80	9.1	35/71 (49)
F_1	M	120	13.7	51/71 (72)
F_1	M	160	16.6	49/71 (69)
F_1	F	0	0	3/124 (2)
F_1	F	20	2.6	51/120 (43)
F_1	F	30	4.0	52/95 (55)
F_1	F	40	5.2	45/72 (63)
F_1	F	60	8.1	55/71 (77)
F_1	F	80	11.2	60/69 (87)
F_1	F	120	22.3	64/72 (89)
MC	M	0	0	17/123 (14)
MC	M	30	3.5	20/118 (17)
MC	M	40	4.7	20/95 (21)
MC	M	60	7.0	23/72 (32)
MC	M	80	9.4	24/71 (34)
MC	M	120	13.2	37/71 (52)
MC	M	160	16.4	32/71 (45)
MC	F	0	0	10/125 (8)
MC	F	20	2.5	54/119 (45)
MC	F	30	3.8	43/95 (45)
MC	F	40	5.1	31/71 (44)
MC	F	60	7.8	37/72 (51)
MC	F	80	10.0	51/69 (74)
MC	F	120	14.8	56/72 (78)

QUALITY OF EVIDENCE

Strength of Study: Both sexes of two different strains of mice, exposed for their lifespan at several levels by a relevant route of administration.

Weakness of Study: Detailed discussion of the data with statistical analyses was not included; general trends were reported. The nature and extent of gross and microscopic examination were not reported; however, an examination of the data indicates that it was probably extensive.

Overall Adequacy: Adequate

[a]Source: Littlefield et al., 1984

[b]F_1 = hybrids from crossing BALB/c males to C57BL females; MC = monohybrid cross offspring of mating F_1 females with F_1 males

[c]Dose in mg/kg/day calculated from data provided by investigators

TABLE 5-2

Incidence of Harderian Gland Adenomas in Two Strains of Mice Given 100% Benzidine Dihydrochloride in Drinking Water for ~33 Months[a]

Strain[b]	Sex	Concentration (ppm)	Corresponding Dose (mg/kg/day)[c]	Tumor Incidence (%)
F_1	M	0	0	6/126 (5)
F_1	M	30	3.7	21/119 (18)
F_1	M	40	4.7	19/96 (20)
F_1	M	60	7.5	18/71 (26)
F_1	M	80	9.1	16/71 (23)
F_1	M	120	13.7	13/71 (19)
F_1	M	160	16.6	18/71 (25)
F_1	F	0	0	5/124 (4)
F_1	F	20	2.6	22/120 (18)
F_1	F	30	4.0	22/95 (23)
F_1	F	40	5.2	21/72 (29)
F_1	F	60	8.1	12/72 (16)
F_1	F	80	11.2	13/72 (18)
F_1	F	120	22.3	8/72 (11)
MC	M	0	0	10/123 (8)
MC	M	30	3.5	20/118 (17)
MC	M	40	4.7	17/95 (18)
MC	M	60	7.0	13/72 (18)
MC	M	80	9.4	13/71 (19)
MC	M	120	13.2	16/71 (23)
MC	M	160	16.4	14/71 (20)
MC	F	0	0	6/125 (5)
MC	F	20	2.5	29/119 (24)
MC	F	30	3.8	16/95 (17)
MC	F	40	5.1	17/71 (24)
MC	F	60	7.8	10/72 (14)
MC	F	80	10.0	9/69 (13)
MC	F	120	14.8	9/72 (12)

[a]Source: Littlefield et al., 1983

[b]F_1 = hybrids from crossing Balb/c males to C57BL females; MC = monohybrid cross offspring of mating F_1 females with F_1 males

[c]Dose in mg/kg/day calculated from data provided by investigators

[d]Incidences were reported as %; the incidence as number of mice with tumors/number of mice examined was calculated from the percentages and from the number of mice examined.

TABLE 5-3

Incidence of Uterine Angiomas in Two Strains of Female Mice Given 100% Benzidine Dihydrochloride in Drinking Water for ~33 Months[a]

Strain[b]	Concentration (ppm)	Corresponding Dose (mg/kg/day)[c]	Tumor Incidence (%)[d]
F_1	0	0	4/124 (3)
F_1	20	2.6	5/120 (4)
F_1	30	4.0	4/95 (4)
F_1	40	5.2	5/72 (7)
F_1	60	8.1	4/72 (6)
F_1	80	11.2	5/72 (7)
F_1	120	22.3	10/72 (14)
MC	0	0	3/125 (2)
MC	20	2.5	1/119 (1)
MC	30	3.8	3/95 (3)
MC	40	5.1	2/71 (3)
MC	60	7.8	5/72 (7)
MC	80	10.0	7/69 (10)
MC	120	14.8	5/72 (7)

[a]Source: Littlefield et al., 1983

[b]F_1 = hybrids from crossing Balb/c males to C57BL/6 females; MC = monohybrid cross offspring of mating F_1 females with F_1 males

[c]Dose in mg/kg/day calculated from data provided by investigators

[d]Incidences were reported as %; the incidence as the number of mice with tumors/number of mice examined was calculated from the percentages and the number of mice examined.

Frith et al. (1979, 1980) exposed groups of male and female weanling mice (Table 5-4) to drinking water containing 0, 30, 60, 120, 200 or 400 ppm benzidine dihydrochloride for 40, 60 or 80 weeks. After dosing was completed the mice were sacrificed for extensive gross and microscopic examination of a wide variety of organs and tissues. Mice that died before 30 weeks (12 females and 22 males) were not included in the analysis; these mice had no liver lesions. Frith et al. (1979) observed changes in the ratio of hepatocellular carcinomas to adenomas with length of administration and dose level in male and female mice. The ratio of hepatocellular carcinomas to adenomas increased with increased dose and longer length of administration in female mice. The ratio of hepatocellular carcinomas increased with increased length of administration but not with dose in male mice. Because of the time of appearance of liver cell lesions, Frith et al. (1980) suggested a sequential morphogenesis from cellular alteration to adenoma to carcinoma. Frith et al. (1979, 1980) also reported that the incidence of pulmonary metastases increased with increased length of administration.

Frith and Dooley (1976) gave groups of 24 male and 24 female Balb/c, 3-week-old mice benzidine dihydrochloride in drinking water at levels of 0, 50, 150, 300 and 500 ppm (Table 5-5). After 8, 19 and 39 weeks of exposure, groups of 4, 4 and 16 animals of each sex, respectively, were killed for comprehensive gross and microscopic examination of a wide variety of organs and tissues. The incidence of tumors in the 300 and 500 ppm groups at 8 or 19 weeks or in the control, 50 or 100 ppm groups was not reported. Presumably, no tumors were found in these groups at these times. Liver tumors were observed in 300 and 500 ppm mice at 39 weeks; the incidence is presented in Table 5-5. Since 16 males and 16 females from each group were

TABLE 5-4

Incidence of Liver Tumors in a Drinking Water Study of Pure Benzidine Dihydrochloride in Mice[a,b]

Sex	Concentration (ppm)	Duration of Treatment and Study (weeks)	Tumor Type	Tumor Incidence (%)
F	0	40	adenoma	0/96 (0)
F	30	40	adenoma	2/195 (1)
F	60	40	adenoma	1/144 (1)
F	120	40	adenoma	2/100 (2)
F	200	40	adenoma	7/100 (7)
F	400	40	adenoma	13/55 (24)
F	0	60	adenoma	1/96 (1)
F	30	60	adenoma	7/146 (5)
F	60	60	adenoma	9/106 (8)
F	120	60	adenoma	17/114 (15)
F	200	60	adenoma	15/121 (12)
F	400	60	adenoma	2/79 (3)
F	0	80	adenoma	0/95 (0)
F	30	80	adenoma	9/86 (10)
F	60	80	adenoma	11/85 (13)
F	120	80	adenoma	5/69 (7)
F	200	80	adenoma	1/17 (6)
F	400	80	adenoma	0/7 (0)
M	0	40	adenoma	0/99 (0)
M	30	40	adenoma	0/199 (0)
M	60	40	adenoma	0/143 (0)
M	120	40	adenoma	1/100 (1)
M	200	40	adenoma	2/102 (2)
M	400	40	adenoma	1/55 (2)
M	0	60	adenoma	0/96 (0)
M	30	60	adenoma	2/142 (1)
M	60	60	adenoma	3/95 (3)
M	120	60	adenoma	9/98 (9)
M	200	60	adenoma	6/90 (7)
M	400	60	adenoma	7/49 (14)
M	0	80	adenoma	2/91 (2)
M	30	80	adenoma	2/85 (2)
M	60	80	adenoma	6/90 (7)
M	120	80	adenoma	11/89 (12)
M	200	80	adenoma	5/40 (13)
M	400	80	adenoma	4/37 (11)

TABLE 5-4 (cont.)

Sex	Concentration (ppm)	Duration of Treatment and Study (weeks)	Tumor Type	Tumor Incidence (%)
F	0	40	carcinoma	0/96 (0)
F	30	40	carcinoma	0/195 (0)
F	60	40	carcinoma	0/144 (0)
F	120	40	carcinoma	1/100 (1)
F	200	40	carcinoma	4/100 (4)
F	400	40	carcinoma	10/55 (19)
F	0	60	carcinoma	1/96 (1)
F	30	60	carcinoma	3/146 (2)
F	60	60	carcinoma	7/106 (7)
F	120	60	carcinoma	33/114 (29)
F	200	60	carcinoma	86/121 (71)
F	400	60	carcinoma	72/79 (91)
F	0	80	carcinoma	0/95 (0)
F	30	80	carcinoma	12/86 (14)
F	60	80	carcinoma	32/85 (38)
F	120	80	carcinoma	60/69 (87)
F	200	80	carcinoma	15/17 (88)
F	400	80	carcinoma	6/7 (86)
M	0	40	carcinoma	0/99 (0)
M	30	40	carcinoma	1/199 (1)
M	60	40	carcinoma	0/145 (0)
M	120	40	carcinoma	0/100 (0)
M	200	40	carcinoma	1/102 (1)
M	400	40	carcinoma	1/55 (2)
M	0	60	carcinoma	1/96 (1)
M	30	60	carcinoma	1/142 (1)
M	60	60	carcinoma	4/95 (4)
M	120	60	carcinoma	8/98 (9)
M	200	60	carcinoma	11/90 (12)
M	400	60	carcinoma	12/49 (24)
M	0	80	carcinoma	0/91 (0)
M	30	80	carcinoma	5/85 (6)
M	60	80	carcinoma	7/90 (8)
M	120	80	carcinoma	16/89 (18)
M	200	80	carcinoma	10/40 (25)
M	400	80	carcinoma	23/37 (62)

TABLE 5-4 (cont.)

Sex	Concentration (ppm)	Duration of Treatment and Study (weeks)	Tumor Type	Tumor Incidence (%)
F	0	40	carcinoma	0/96 (0)
F	30	40	carcinoma	0/195 (0)
F	60	40	carcinoma	0/144 (0)
F	120	40	carcinoma	1/100 (1)
F	200	40	carcinoma	4/100 (4)
F	400	40	carcinoma	10/55 (19)
F	0	60	carcinoma	1/96 (1)
F	30	60	carcinoma	3/146 (2)
F	60	60	carcinoma	7/106 (7)
F	120	60	carcinoma	33/114 (29)
F	200	60	carcinoma	86/121 (71)
F	400	60	carcinoma	72/79 (91)
F	0	80	carcinoma	0/95 (0)
F	30	80	carcinoma	12/86 (14)
F	60	80	carcinoma	32/85 (38)
F	120	80	carcinoma	60/69 (87)
F	200	80	carcinoma	15/17 (88)
F	400	80	carcinoma	6/7 (86)
M	0	40	carcinoma	0/99 (0)
M	30	40	carcinoma	1/199 (1)
M	60	40	carcinoma	0/143 (0)
M	120	40	carcinoma	0/100 (0)
M	200	40	carcinoma	1/102 (1)
M	400	40	carcinoma	1/55 (2)
M	0	60	carcinoma	1/96 (1)
M	30	60	carcinoma	1/142 (1)
M	60	60	carcinoma	4/95 (4)
M	120	60	carcinoma	8/98 (9)
M	200	60	carcinoma	11/90 (12)
M	400	60	carcinoma	12/49 (24)
M	0	80	carcinoma	0/91 (0)
M	30	80	carcinoma	5/85 (6)
M	60	80	carcinoma	7/90 (8)
M	120	80	carcinoma	16/89 (18)
M	200	80	carcinoma	10/40 (25)
M	400	80	carcinoma	23/37 (62)

TABLE 5-4 (cont.)

QUALITY OF EVIDENCE

Strengths of Study:	Both sexes of one species were dosed at several levels for durations up to the lifespan of the organism by a relevant route of administration. The mice were subjected to extensive histopathological examination
Weakness of Study:	Only one species (mice) were treated
Overall Adequacy:	Adequate

[a]Source: Frith et al., 1979, 1980

[b]F_1 (C57BL females x Balb/c males) and MC (F_1 females x F_1 males) mice combined. Incidence data for the two strains were combined because there were no statistical differences between the two strains.

TABLE 5-5

Incidence of Liver Tumors in Balb/c Mice Treated With Benzidine Dihydrochloride (Purity NR) in Drinking Water for 39 Weeks[a,b]

Sex	Concentration (ppm)	Tumor Type	Tumor Incidence
M	300	benign hepatoma	1/12
M	300	carcinoma	0/12
F	300	benign hepatoma	1/9
F	300	carcinoma	0/9
M	500	benign hepatoma	3/7
M	500	carcinoma	0/7
F	500	benign hepatoma	0/12
F	500	carcinoma	3/12

QUALITY OF EVIDENCE

Strengths of Study: .Both sexes of one species (mice) were tested at several doses by a relevant route of administration. Comprehensive gross and microscopic examination of organs and tissues was performed.

Weakness of Study: Mice were not dosed for their lifespan. The data were not subjected to statistical analysis. Control data not reported.

Overall Adequacy: Inadequate

[a]Source: Frith and Dooley, 1976

[b]The duration of treatment was equal to the duration of the study.

sacrificed at 39 weeks and since the occurrence of spontaneous death was not reported, the reason for the discrepancy between the number of mice examined and the number killed is not apparent.

In a series of experiments, Vesselinovitch et al. (1975) administered 50 or 100 ppm benzidine dihydrochloride in the diet to groups of 50 male and 50 female B6C3F1 mice for 90 weeks. Presumably, a control group was maintained on a diet without benzidine dihydrochloride, but this was not explicitly reported. The authors reported that complete autopsies were performed but did not provide sufficient detail for a determination of the adequacy of the gross and microscopic examination. The MTD was estimated by pretesting the mice. The authors did not always provide sufficient information to report tumor incidence, as indicated in Table 5-6. Vesselinovitch et al. (1975) also conducted gavage studies in mice. Groups of 75 male and 75 female B6C3F1 mice were gavaged twice weekly with doses of 0.5 or 1.0 mg benzidine/mouse at each intubation for 90 weeks. These intermittent doses were considered to be equivalent to the weekly intake of benzidine dihydrochloride by the mice exposed continuously to 50 and 100 ppm in the diet. The incidence of tumors is presented in Table 5-6. Higher incidences of liver tumors and Harderian gland tumors were observed in the mice treated continuously in the diet compared with the mice receiving intermittent equivalent gavage doses. The incidence of lung adenomas, however, was higher in the mice treated by gavage than in the mice treated by dietary administration.

In addition to the tumors presented in Table 5-6, a marginally increased incidence of lymphoreticular tumors were observed in the treated mice compared with controls. (This was the only mention of controls in the entire report.) The incidences in individual groups were low; therefore, Vesselinovitch et al. (1975) reported the combined incidences regardless of dose

TABLE 5-6

Incidence of Tumors in B6C3F1 Mice Treated Orally with Benzidine Dihydrochloride (Certified Grade)*

Sex	Concentration or Dose	Duration of Treatment (weeks)	Duration of Study (weeks)	Vehicle or Physical State	Target Organ	Tumor Type	Tumor Incidence
M	50 ppm	90	90	dietary	liver harderian gland lung	carcinoma NR adenomas	3/50 9/50 2/50
M	100 ppm	90	90	dietary	liver harderian gland lung	carcinoma NR adenomas	11/50 18/50 3/50
F	50 ppm	90	90	dietary	liver harderian gland lung	carcinoma NR adenomas	13/50 3/50 2/50
F	100 ppm	90	90	dietary	liver harderian gland lung	carcinoma NR adenomas	32/50 3/50 2/50
M	0.5 mg twice weekly	90	90	NR/gavage	liver harderian gland lung	carcinoma NR adenomas	3/75 2/75 12/75
M	1.0 mg twice weekly	90	90	NR/gavage	liver harderian gland lung	carcinoma NR adenomas	12/75 11/75 19/75
F	0.5 mg twice weekly	90	90	NR/gavage	liver harderian gland lung	carcinoma NR adenomas	4/75 2/75 5/75

TABLE 5-6 (cont.)

Sex	Concentration or Dose	Duration of Treatment (weeks)	Duration of Study (weeks)	Vehicle or Physical State	Target Organ	Tumor Type	Tumor Incidence
F	1.0 mg twice weekly	90	90	NR/gavage	liver harderian gland lung	carcinoma NR adenomas	17/75 5/75 4/75
M	150 ppm	39 54 84	84	dietary	liver liver liver	carcinoma carcinoma carcinoma	35/50 25/50 22/50

QUALITY OF EVIDENCE

Strengths of Study: Both sexes of one species (mice) were dosed at several levels by a relevant route of administration over their lifespan. The MTD was determined by pretesting.

Weakness of Study: Tumor incidence was not always expressed as number of tumors/number of animals treated. Insufficient information was given to assess the adequacy of the histopathologic examination. Only x^2 test was used to determine statistical significance between various experimental groups. With the exception of one table, no data for controls were reported.

Overall Adequacy: Limited

*Source: Vesselinovitch et al., 1975

NR = Not reported

0738p

04/12/86

or sex to be 19/200 (9.5%) for the continuously (diet) exposed mice, 17/300 (5.7%) for the intermittently (gavage) exposed mice and 4/194 (2.0%) for the controls.

In another experiment (Vesselinovitch et al., 1975), groups of 50, 6-week-old male mice were exposed to benzidine dihydrochloride in the diet at 150 ppm for 39, 54 or 84 weeks and were killed at 90 weeks of age. As seen from Table 5-6, there was a higher incidence of liver carcinomas in the mice treated for shorter time periods. The reason for this observation was not apparent.

Vesselinovitch et al. (1975) also reported the total liver tumor (benign and malignant) incidences in male mice fed 150 ppm benzidine dihydrochloride from weeks 6-45 of age and subsequently fed diets without benzidine for 45, 60, 75 and 90 weeks as 16, 40, 62 and 70%, respectively. The authors concluded that "once carcinogenesis has been initiated, the neoplastically committed cells expressed their malignant character once a sufficient observational period has been allowed."

Vesselinovitch et al. (1975) also investigated the role of age at the time of treatment on tumor induction. Male and female 7- to 27-day-old B6C3F1 mice were treated daily with 30 µg benzidine dihydrochloride in 0.05 mℓ distilled water by gavage for 33 weeks. Liver tumors were observed in 59/89 males (66%) and in 0/82 females. Daily treatment of adult mice by gavage with 100 µg benzidine dihydrochloride in 0.05 mℓ distilled water from age 42-62 weeks resulted in liver tumors in 2/50 males (4%) and 0/50 females.

Vesselinovitch et al. (1979) exposed groups of 44-50 male and 43-50 female B6C3F1 mice to a dietary concentration of 150 ppm of benzidine dihydrochloride during the prenatal, preweaning and adult periods as well as

combinations of these periods as indicated in Table 5-7. A group of 98 males and 100 females given food without benzidine dihydrochloride served as controls. Hepatocellular tumor incidences for each period are reported in Table 5-7. There was an increased incidence of liver tumors in males but not in females treated with benzidine dihydrochloride during the prenatal or preweaning exposure period. A high incidence of liver tumors, however, was observed in females exposed to benzidine throughout the prenatal and preweaning periods. Increased incidences of liver tumors occurred in both sexes when the mice were treated from weaning to the 90th week. The females were more susceptible to the hepatocarcinogenic effect of benzidine dihydrochloride during this period than were the males. Almost all mice of both sexes dosed during the prenatal, preweaning and adult periods developed liver tumors. Vesselinovitch et al. (1979) also reported that the metastatic rate and percent transplantability were 18/59 and 38% in mice exposed to benzidine dihydrochloride during the preweaning period.

Osanai (1976) fed groups of 3-8 ICR/JCL male mice 0.1% (1000 ppm) benzidine of unspecified purity in their diet for up to 14 months. After 6 months of treatment, 8/8 treated mice had hepatomas as compared with 0/5 in controls. After 12 months of exposure, 3/3 mice had hepatomas as compared with 2/12 mice in the control group. After 14 months of exposure, 7/7 treated mice had hepatomas as compared with 5/18 control mice.

Ikuyama et al. (1978) gave male ICR/JCL mice dietary benzidine at a concentration of 0.05% (500 ppm) for 3-9 weeks. The mice were observed for an additional 23-29 weeks. The treated mice were divided into four groups. Group 1 was allowed to exercise freely; group 2 was forced to exercise during treatment; group 3 was forced to exercise for 26 weeks after treatment ceased; and group 4 was given the benzidine containing diet but was

TABLE 5-7

Incidence of Liver Tumors in B6C3F1 Mice Fed Benzidine Dihydrochloride of Unspecified Purity During Various Developmental Periods[a]

Sex	Concentration (ppm)	Treatment Period	Tumor Incidence (%)
M	150	prenatal[b]	17/55 (31)
M	150	preweaning[c]	62/65 (95)
M	150	adult[d]	25/44 (57)
M	150	prenatal and preweaning[b,c]	49/49 (100)
M	150	prenatal, preweaning and adult[b,c,d]	50/50 (100)
M	0	none	1/98 (1)
F	150	prenatal[b]	2/62 (3)
F	150	preweaning[c]	2/43 (5)
F	150	adult[d]	48/50 (96)
F	150	prenatal and preweaning[b,c]	12/48 (25)
F	150	prenatal, preweaning and adult[b,c,d]	47/50 (94)
F	0	none	0/100 (0)

[a]Source: Vesselinovitch et al., 1979

[b]Given to dams from day 12 to delivery

[c]Given to dams with litters from delivery to weaning

[d]Given to offspring from weaning through 90 weeks

permitted no exercise. The incidences of hepatoma for groups 1, 2, 3 and 4 were 50, 57.9, 72.2 and 82.1%, respectively. From these results the authors concluded that exercise had a repressive effect on the incidence of benzidine induced hepatoma.

Miyakawa and Yoshida (1980) inserted glass beads into the bladders of 5-week-old female ICR mice. One week later a group of 60 of these mice were given benzidine in the diet at a concentration of 0.2% (2000 ppm) for 20 weeks. A 40- to 43-week observation period followed dosing. A group of 30 mice given basal diets without benzidine served as controls. Gross and histopathological examination of the urinary bladder and liver as well as a number of other organs was performed. Survival of the mice was not affected up to week 40, but at 60 weeks only 19.3% of the benzidine-exposed mice survived, compared with 65.5% survival in the controls. The body weights of benzidine treated mice (39.49$\pm$7.74 g) were significantly reduced compared with controls (52.77$\pm$17.00 g). The liver weight relative to body weight was significantly greater in benzidine-treated mice (0.31$\pm$0.09) compared with controls (0.05$\pm$0.02). No bladder tumors were observed; however, the incidence of hepatomas was 34/41 compared with 0/20 in controls.

Griswold et al. (1968) exposed groups of twenty 40-day-old female Sprague-Dawley rats to a total of 12, 25, 35 and 50 mg benzidine/rat by gavage in sesame oil, given in 10 doses at 3-day intervals over a 30-day treatment period. Controls were dosed with sesame oil alone. The rats were observed for 9 months before sacrifice. Gross lesions were recorded, and the pituitary, adrenals, kidneys, spleen and liver were weighed. In addition to diseased tissues, the mammary, intestinal tract, pituitary, liver, ovaries and adrenals were prepared for histological examination. The 12, 25 and 50 mg dosage group had carcinomas and adenomas of the mammary glands (Table 5-8). There were no survivors in the 35 mg group at 9 months.

TABLE 5-8

Incidence of Mammary Gland Tumors in Female Sprague-Dawley Rats Treated by Gavage With Benzidine (Pure) in Sesame Oil for 30 Days and Observed for 9 Months[a]

Dose[b] (mg/rat)	Tumor Type	Tumor Incidence (%)
50	carcinoma	4/5 (80)
35	no survivors at 9 months	NA
25	carcinoma	7/9 (78)
12	carcinoma, adenoma	5/10 (50)
0	carcinoma, adenoma, hyperplasia	5/132 (4)

QUALITY OF EVIDENCE

Strengths of Study: Several doses were administered by a relevant route of administration (oral); the MTD was estimated through pretests.

Weakness of Study: Only female rats of one species were used; dosing occurred at 3-day intervals over a 30-day period, which is a period substantially less than the lifespan of a rat; histopathology was not comprehensive and the data were not subjected to statistical analysis.

Overall Adequacy: Limited

[a]Source: Griswold et al., 1968

[b]10 doses at 3-day intervals; dose reported = total dose

NA = Not applicable

The authors noted spontaneous regression of some of the palpable masses; therefore, some tumors were not present at autopsy.

Boyland et al. (1954) gave groups of 10 female Wistar rats diets with or without tryptophan containing 0.017% benzidine by weight (170 ppm) until a tumor appeared or death occurred. Diets with and without tryptophan were used to test the hypothesis that addition of tryptophan would change the metabolism of aromatic amines so that more proximate carcinogens would be formed. Control rats were also treated with diets not containing benzidine, but tumor data for the controls were not reported. Of the 10 rats given dietary benzidine without the tryptophan supplement, 2 developed cancer (1 hepatoma and 1 carcinoma of the bile duct after 125 and 178 days of treatment, respectively). The other 8 rats in this treatment group died from other causes. Of the 7 rats given dietary benzidine with a tryptophan supplement, 2 developed cancers (1 carcinoma of the liver and 1 carcinoma of the bile duct after 202 and 424 days of treatment, respectively). The other 5 rats in this treatment group died of other causes.

Groups of 30 male and 30 female 9-week-old Syrian golden hamsters were fed a diet containing 1000 ppm benzidine or benzidine dihydrochloride for life (Saffiotti et al., 1967; Sellakumar et al., 1969). A control group was maintained as well. Cholangiomas and benign and malignant hepatomas were found at incidences reported in Table 5-9. No liver tumors were observed in male and female controls.

Bonser (1959) administered benzidine to 13 rabbits at an oral dose of 20 mg/week for 3.5 years and found one urinary bladder tumor and one gall bladder tumor. Control data were not reported.

Marhold et al. (1967) gave one dog an oral dose of pure benzidine at 5 mg/kg/day for 6 years. No tumors were observed.

TABLE 5-9

Incidence of Liver Tumors in Syrian Golden Hamsters Given 1000 ppm Benzidine or the Dihydrochloride (Certified Grade) in the Diet for the Lifespan[a]

Sex	Tumor Type	Tumor Incidence[b]
M	cholangioma (mostly malignant)	19/22[c]
F	cholangioma (mostly malignant)	6/26[c]
M	benign and malignant hepatoma	12/22[c]
F	benign and malignant hepatoma	3/26[c]
M	cholangioma (mostly malignant)	10/20[d]
F	cholangioma (mostly malignant)	12/27[d]
M	benign and malignant hepatoma	7/20[d]
F	benign and malignant hepatoma	4/27[d]

QUALITY OF EVIDENCE

Strengths of Study: Both sexes of one species were dosed by a relevant route of administration for their lifespan

Weakness of Study: Only one dose was used

Overall Adequacy: Limited

[a]Source: Saffiotti et al., 1967; Sellakumar et al., 1969

[b]Control incidence = 0/30 in males and 0/30 females

[c]Dosed with benzidine

[d]Dosed with benzidine dihydrochloride

Spitz et al. (1950) fed a group of seven (one male, six females) "full grown" mongrel dogs (~25 pounds or 11 kg) capsules containing 200 mg of benzidine, 6 days/week for 15 months and 300 mg, 6 days/week thereafter for the next 45 months (TWA = 236 mg/kg/day). The dogs were observed for at least 10 years following the beginning of the study. Periodic cytoscopic examination of the urinary bladder of the female dogs revealed one dog with an epidermoid carcinoma of the bladder at 7 years. As discussed by Bonser et al. (1956), Spitz (n.d.) also found bladder tumors in two more females after 8 and 10 years. The male dog was not cytoscoped (Spitz et al., 1950). Bonser et al. (1948) found no bladder tumors in dogs fed a total of 700 g over 5 years

Deichmann et al. (1965) dosed a group of six, 6- to 12-month-old, female purebred beagle dogs with 1.0 mg/kg benzidine in capsules, 5 days/week for ~3 years. Gross and microscopic examination of the bladder was performed; however, whether or not other organs or tissues were examined as well was not specified. No bladder tumors were observed in any of the dogs. The results of these studies in dogs indicate that induction of bladder tumors in dogs may require a long latency period.

Zabezhinskii (1970) exposed a group of 48 noninbred albino rats of both sexes, weighing 100-120 g, to benzidine by inhalation at a concentration of 10-20 mg/m^3, 4 hours/day, 5 times/week for 28 months. Control rats were exposed to "pure air." Rats were examined histologically, but the nature and extent of the examination was not specified. Five of 28 exposed rats surviving until $\geq$13 months developed myeloid leukemia after 13, 22, 24, 25 and 28 months of the experiment. In the mammary gland, fibroadenomas occurred in 2/28 rats, and carcinomas occurred in 2/28 rats; one of the mammary gland carcinomas was found in a male rat. In addition, 1/28 rats

had a hepatoma. The authors reported that 8/28 rats had tumors; therefore, (presumably) more than one tumor type was found in some of the rats. The incidence of tumors in control rats was 2/21 surviving until ≥13 months. Both of the control rats had mammary adenomas that developed at 22 and 26 months.

Zabezhinskii (1967) fed an unspecified strain of rats 10 mg of benzidine at 1-month intervals for 7 months by intratracheal tube. After 8 months of the experiment, 6 male rats of a group of 14 rats of unspecified sex had carcinomas of the mammary gland, 4 female rats of the same group of 14 rats had mammary gland carcinomas and 1 rat of an unspecified sex had a liver cholangiocarcinoma.

Numerous sources have reviewed the carcinogenic effect of benzidine by subcutaneous, intramuscular and intraperitoneal routes of administration (IARC, 1972, 1982; U.S. EPA, 1980a). Several studies have reported tumors remote to the site of subcutaneous injection including the following: intestinal tumors in rats (Pliss et al., 1973); mammary tumors in rats (Steinhoff, 1974, 1981); Zymbal gland tumors in rats (Pliss and Vol'fson, 1972); hepatomas in mice (Baker, 1950; Bonser et al., 1956; Prokof'eva, 1971) and rats (Spitz et al., 1950); adenocarcinomas in mice (Prokof'eva, 1971); lymphoma in mice (Bonser et al., 1956); and tumors of the sebaceous glands of the auditory canal in rats (Spitz et al., 1950). Tumors of the mammary gland and Zymbal gland were observed in rats after intraperitoneal injection of benzidine (Morton et al., 1981).

5.1.2. Human Studies. Numerous epidemiology studies that link exposure to benzidine with the development of bladder cancer have been performed. Zavon et al. (1973) conducted a prospective study of a cohort of men occupationally exposed to benzidine during its manufacture; there were no controls.

The investigators considered inhalation as the primary route of exposure. The level of exposure (taken from various locations in the plant) ranged from <0.005-17.6 mg/m³. Although the sample period was limited, the investigators were assured that "conditions were reasonably representative of the conditions that had prevailed" previously. Zavon et al. (1973) also measured urinary benzidine levels; at the end of the workshift, the mean urinary level was ~0.035 mg/ℓ. From this level, U.S. EPA (1980a) estimated a mean total accumulated dose of 130 mg/kg. The mean duration of exposure was 11.46 years. Thirteen members of the 25-member cohort had bladder cancer. These 13 workers had a mean duration of exposure of 13.6 years, while the 12 workers without bladder cancer had a mean exposure duration of 8.91 years. Four renal tumors also were found in three workers. Metastases to the neck and abdomen were found in two workers with bladder cancer. In workers with bladder tumors, the mean age at first tumor and induction period was 45.62 and 16.62 years, respectively. The authors noted that 11/13 workers with bladder tumors had "no known contact with β-naphthylamine, though some were exposed to dichlorobenzidine and o-toluidine." Thus, the authors concluded that at least 11/13 tumor cases were attributed to benzidine exposure. The authors reported that two survivors refused to participate in the study and workers exposed to benzidine before 1958 could not be located. Thus, the number of tumors in workers occupationally exposed to benzidine may be underestimated.

The Zavon et al. (1973) study reported adequately the level and duration of exposure; however, exposures to other chemicals occurred. The number of tumors expected was not reported; therefore, relative risk could not be calculated. A rough estimate of relative risk can be derived for benzidine using the "Third National Cancer Survey: Incidence Data." Using this data a

relative risk of ~100 can be estimated. Therefore, despite the fact that smoking and exposure to other chemicals were not controlled for in the Zavon study, because of the high potency of benzidine these may not be major contributing factors (NCI, 1975).

Tsuchiya et al. (1975) conducted a study to determine the incidence of bladder cancer among workers (sex not reported) who were involved in the production and use of benzidine. There was no control population and the cohort also included workers in other populations. Of the 1015 workers examined, 72 had bladder cancer (21 were fatal). The number of bladder cancer cases in 346 production workers was 61 (17.6%). In workers who used benzidine, 11/669 workers (1.6%) developed bladder cancer. The mean$\pm$SD latency period for tumor development was ~16.2$\pm$7.38 years. Relative risk could not be calculated because the number of tumors expected was not reported. The authors commented that bias existed in the sample because retired workers who may have had tumors were not included.

Meigs et al. (1986) conducted a 30-year follow-up study on the incidence of bladder tumors among workers in Connecticut exposed to benzidine. In this study records from the Connecticut Tumor Registry were used to determine the cancer incidence in a cohort of workers (n=984) at a benzidine manufacturing facility. These findings were compared with the incidence of cancer for the Connecticut population. There was a statistically significant excess of bladder tumors among male cohort members (Table 5-10) which was confined to those males with the highest estimated level of benzidine exposure. Exposure was estimated as high, medium or low according to the length of individual exposure to benzidine ($\geq$2 years = high; 6 months to 2 years = medium; $\leq$6 months = low). Among the 830 males, a total of 48 first primary cancers were reported (see Table 5-10); of these 48, eight cases of

TABLE 5-10

Observed vs. Expected Numbers of Incident Cancers and SIR by Tumor Site[a]

Site[b]	Observed	Expected	SIR
All sites except non-melanoma skin	48	35.9	134
Urinary bladder	8	2.3	343[c]
Stomach	3	1.6	185
Colon	5	3.4	145
Trachea, bronchus, lung	11	7.3	150
Prostate gland	5	3.0	165
Other unspecified	16	18.2	88

[a]Source: Meigs et al., 1986

[b]Males only

[c]$p<0.01$

bladder cancer were found. Benzidine exposure was present in 7 (6 high; 1 medium) of the 8 cases of bladder cancer. No significantly elevated risks were found for cancers at other anatomic sites in men or at any anatomic site for women; nor was there any pattern of increasing risk with increasing benzidine exposure for site other than bladder. In addition, the elevated bladder cancer was greater for men first employed during the earliest years of the plant, namely, 1945-1949. Smoking histories were available for 21 of the 48 primary cancers; of the 8 cases of bladder cancer, 3 were long-term cigarette smokers.

Case et al. (1954) conducted a study of 4622 male British workers occupationally exposed to benzidine, aniline, α-naphthylamine or β-naphthylamine. The authors reported 10 deaths that were attributed to benzidine exposure only (total number of benzidine-exposed workers unspecified). The expected number of deaths based on rates for the whole male population of England and Wales was 0.72. In addition to these cases were 24 cases of nonfatal bladder tumors. The authors noted a tendency to underestimate the number of deaths attributed to occupational exposure because of failure to track all cases. Most cases were observed in workers manufacturing benzidine, but bladder tumors were found in workers using benzidine as well.

Mancuso and El-Attar (1966, 1967) noted 18 deaths from bladder and kidney cancer in a cohort of 640 workers occupationally exposed to benzidine and β-naphthylamine. Bladder cancer mortality in benzidine workers exposed to benzidine and β-naphthylamine was 30 times higher than expected based on Ohio mortality figures for males.

In an article that was primarily written to discuss a surveillance system for discovery of bladder tumors in dyestuff workers, Yamaguchi et al. (1982) reported 9 cases of bladder tumors in 244 workers monitored. Six of these cases were attributed to benzidine exposure alone.

Morinaga et al. (1982) studied a population of 3322 Japanese workers exposed to benzidine or ß-napthylamine or both. Of these, 244 workers died from primary urogenital cancer. Of this group, 11 workers also had tumors at other sites. The study cohort had a statistically significant ($p<0.05$) increased incidence of liver, gall bladder and bile duct cancer as compared with a control population of 177 male bladder cancer patients.

Budnick et al. (1984) reported a statistically significant ($p<0.025$) increased number of bladder cancer deaths in white males and increases of other types of cancer during the 1970s in the general population of areas surrounding the Drake Superfund site in Clinton County, PA. This site was contaminated by ß-naphthylamine, benzidine and benzene.

Segnan and Tanturri (1976) observed an excess of bladder cancer in men in an area where an epidemic of bladder cancer was observed in workers who used ß-naphthylamine and benzidine in the past. Nineteen bladder cancer cases were observed as compared with an expected incidence of 9.18 ($p<0.01$). The relative risk was 207% ($p<0.01$).

Qi et al. (1983) monitored 374 benzidine-exposed workers with urinary exfoliative cytology according to Papanicolaou's criteria. Cancer cells were found in the urine of five workers.

Forni et al. (1972) reported 17 bladder tumors in a cohort of 858 benzidine dyestuff workers. The maximum latency period was 16 years after exposure was terminated.

Numerous case reports were cited in the available literature concerning the occurrence of bladder cancer in dyestuff industry workers. These reports provide qualitative evidence of the carcinogenic effect of benzidine. Several sources that contain reviews of these studies are IARC (1972, 1982), U.S. EPA (1980a,b), ACGIH (1980) and NIOSH (1978). Haley (1975),

Hamilton and Hardy (1974) and Parkes (1976) reviewed the history of benzidine induced cancer in humans. Haley (1975) also discussed the international dimensions of benzidine induced cancer. Several early reports came from industrial settings in which workers were exposed to benzidine alone. Aboulker and Smagghe (1953) reported 21 cases of bladder tumors in workers at a dyestuff plant. In two of these cases, the workers were exposed to benzidine alone. Uebelin and Pletscher (1954) observed 100 cases of urinary tract tumors in Swiss dyestuff workers. Twenty of these workers were exposed to benzidine alone. Duration of exposure was ~11.6 years (range, 1-29 years). The mean induction period was 14.8 years (range 5-29 years). Billiard-Duschesne (1960) described 12 bladder cancer cases using workers at a plant where the only aromatic amine manufactured was benzidine. Goldwater et al. (1965) reported 17 cases of bladder cancer in a population of 76 men occupationally exposed to benzidine only for up to 33 years, and commented that this incidence may be deceptively low since many workers quit their jobs after the first examination. Ferber et al. (1976) reported that 36 cases of bladder tumors occurred in workers exposed to benzidine alone during the period of 1930-1975.

Occasionally a report indicated tumor induction at sites other than the bladder in benzidine exposed workers. Maltoni and Ghetti (1964) reported four cases of kidney and ureter cancer attributable to benzidine exposure. Uebelin and Pletscher (1954) also observed lung, rectum and prostate cancer, which was thought to be linked to benzidine exposure.

5.2. MUTAGENICITY

Mutagenicity data for benzidine before 1982 have been summarized in U.S. EPA (1980a), IARC (1982) and Waters et al. (1983). According to these reviews, benzidine has been consistently mutagenic in Salmonella typhimurium

TA1538 with metabolic activation from rat (Ames et al., 1973; Anderson and Styles, 1978) and human liver preparations (Haworth and Lawlor, 1978). When tested with or without rat liver metabolic activation, benzidine was negative in the prophage induction test (Speck et al., 1978) and Escherichia coli pol A test (Fluck et al., 1976). In Drosophila melanogaster, benzidine had a mutagenic effect with respect to X-chromosome recessives (visibles and lethals) and RNA genes (Fahmy and Fahmy, 1977). When given intraperitoneally or intragastrically to suckling 14- to 18-day-old mice at doses 15-30% of the LD_{50}, benzidine inhibited DNA synthesis in renal and hepatic cells (Amlacher and Ziebarth, 1979). DNA synthesis was also inhibited in vitro in HeLa cells without activation (Painter, 1978). Benzidine induced UDS in HeLa cells in the presence of a phenobarbital-induced rat liver activation system (Martin et al., 1978) and in rat hepatocytes (Williams, 1978; Brouns et al., 1979). When tested in Chinese hamster V79 cells with rat liver metabolic activation, benzidine induced DNA strand breaks (Swenberg et al., 1976). A dose-related increase in DNA strand breaks in the livers of rats exposed to benzidine in vivo was observed by measurement with the alkaline elution assay. Benzidine (2.5 µg/mL) transformed Syrian hamster embryo cells (Pienta, 1980) and BHK21 C1-13 cells in the presence of an Aroclor 1254-induced rat liver metabolic system (Ashby et al., 1978).

In addition, several reports were located in the available literature that summarized the results obtained in numerous mutagenicity tests. Ashby and Kilbey (1981) reported positive results in the E. coli $RecA^-/PolA^-$ and E. coli Pol A differential killing assays. Negative results were obtained in the inductest with prophage lambda and positive results in a modified inductest in which mutation was assayed by induction of the gal operon. Positive results were reported in nuclear enlargement assays using

HeLa cells and human fibroblasts. DeSerres and Hoffmann (1981) reported that benzidine yielded positive results in assays for mitotic recombination in Saccharomyces cerevisiae strains JD1 and D7, mitotic aneuploidy tests in S. cerevisiae strain D6 and in repair tests with the S. cerevisiae rad mutant. Negative results were obtained in forward mutation tests in S. pombe strain P_1 and reverse mutation assays in S. cerevisiae strain XV185-14C, and mitotic recombination assays in S. cerevisiae D4, T_1 and T_2. Brookes and Preston (1981) reported that benzidine yielded positive results in the following in vitro mammalian assay systems: UDS, sister chromatid exchange, cytogenetics, gene mutation and transformation. Purchase and Ray (1981) summarized the results of in vivo assays. Negative results were obtained in the Drosophila melanogaster sex-linked recessive lethal test and in the sperm abnormality assay. Positive results were reported for an in vivo sister chromatid exchange assay and the micronucleus test.

More recent mutagenicity data are presented in Table 5-11.

5.3. TERATOGENICITY

Very little information concerning the possible teratogenicity of benzidine was located in the available literature. Vesselinovitch et al. (1979) (see Section 5.1.) observed an increased incidence of hepatocellular carcinomas in the male offspring of female mice maintained on a diet containing 150 ppm benzidine dihydrochloride during gestation. Female offspring were not affected.

Budnick et al. (1984) reported no statistically significant increase in any particular type of birth defect or in all types of birth defects in residents in the area surrounding the Drake Superfund site in Clinton County, PA (see Section 5.1.2.). This site was contaminated by β-naphthylamine, benzidine and benzene.

TABLE 5-11

Mutagenicity Testing of Benzidine

Assay	Indicator Organism	Application	Concentration or Dose Range	Activating System	Response	Comment	Reference
Reverse mutation	Salmonella typhimurium TA1538	plate assay	25-100 µg/plate	+ mouse S-9 + hamster S-9 + rat S-9 + pig S-9 + human S-9	+ + - - -	Not tested in absence of metabolic activation	Phillipson and Ioannides, 1983
Reverse mutation	S. typhimurium TA1538	hepatocyte suspension assay	0-500 µM	+ rat hepatocytes	+	Dose-related increase at ≤100 mM; number of revertants remained constant over range of 100-500 µM; not tested in absence of hepatocytes	Bos et al., 1983
Reverse mutation	S. typhimurium TA100	hepatocyte suspension assay	0-500 µM	+ rat hepatocytes	+	Dose-related increase at ≤500 µM; the response was weaker than in TA1538	Bos et al., 1983
Reverse mutation	S. typhimurium TA1538	plate assay	0-250 nmol/ plate	+S-9	+	Dose-related increase at ≤250 nmol/plate; the response was weaker with S-9 than with hepatocytes	Bos et al., 1983
Reverse mutation	S. typhimurium TA100	plate assay	0-250 nmol/ plate	+S-9	-	NC	Bos et al., 1983
Reverse mutation	S. typhimurium TA1538	suspension	0-500 µM	intact hepatocytes	+	Increased maximally to ~400 revertants at ~100 µM and declined steadily to ~300 revertants at ~500 µM	Bos et al., 1983
Reverse mutation	S. typhimurium 1538	suspension	0-500 µM	S-9 from disrupted hepatocytes	+	Increased maximally to ~80 revertants at 100 µM and declined steadily to ~25 revertants at 500 µM	Bos et al., 1983
Reverse mutation	S. typhimurium TA98, TA100	plate assay	25-100 µg/plate	S-9 mix	+	Not tested in absence of S-9	Parodi et al., 1981
Reverse mutation	S. typhimurium TA98, TA100	plate assay	10-40 µg/plate	bovine bladder urothelial cells	+	TA98 only	Oglesby et al., 1983

0738p

06/05/86

TABLE 5-11 (cont.)

Assay	Indicator/ Organism	Application	Concentration or Dose Range	Activating System	Response	Comment	Reference
Reverse mutation	S. typhimurium TA1538 TA1535 TA1537 TA98 TA100	plate assay	10-10,000 µg/plate	±S-9	± - - ± ±	NC	Simmon and Shepherd, 1981
Forward mutation	CHO V79	cell culture	5-20 µg/ml	± bovine bladder urothelial cells	- -	NC	Oglesby et al., 1983
Transformation	BALB/3T3 embryo cells	cell culture	30 µM	none	+	NC	Cortesi et al., 1983
Forward mutation	L5178Y TK+/- mouse lymphoma	cell culture	0-50 µg/ml	+S-9	+	Not tested in absence of S-9	Oberly et al., 1984
Forward mutation	human fibroblasts	in vitro	NR	±S-9	±	Not tested in absence of S-9	Huang and Waters, 1983
Rec assay	Bacillus subtilis H17 rec+; M45 rec-	plate assay	2 mg	±S-9	±	The response in the absence of S-9 was weak.	Kada, 1981
UDS	rat hepatocytes	in vitro	10^{-4} to 2×10^{-8} M	NA	+	NC	Kornbrust and Barfknecht, 1984a,b
UDS	hamster hepatocytes	in vitro	10^{-4} to 2×10^{-8} M	NA	+	NC	Kornbrust and Barfknecht, 1984a,b
DNA repair (Nick translation)	human diploid fibroblasts	in vitro	3 mM	none	-	NC	Snyder and Matheson, 1985
SOS chromotest	E. coli PQ37	liquid medium	NR	+S-9	-	Not tested in absence of S-9	Quillardet et al., 1985
DNA damage (alkaline elution)	male Sprague-Dawley rat liver cells	administered intraperitoneally	1.08 mmol/kg	NA	+	p<0.002	Parodi et al., 1981

TABLE 5-11 (cont.)

Assay	Indicator/ Organism	Application	Concentration or Dose Range	Activating System	Response	Comment	Reference
DNA damage (alkaline elution)	rat hepatocytes	In vitro	0.03-3 mM	NA	+	NC	Sina et al., 1983
DNA damage (alkaline elution)	rat hepatocytes	In vivo by gavage	0.4 mL/100 g bw	NA	+	NC	Bermudez et al., 1982
DNA damage	human diploid fibroblasts	In vitro	3 mM	none	+	NC	Snyder and Matheson, 1985
Micro-nucleus assay	bone marrow of B6C3F1/BR hybrid female mice	Intraperi-toneal injection	88 mg/kg	NA	+	NC	Katz et al., 1981
SCE	bone marrow cells of Swiss male mice	In vivo intraperi-toneal	87.7 and 13.5 mg/kg	NA	+	NC	Parodi et al., 1982
SCE	V79-1K1 lung fibroblasts	NR	NR	+S-9	+	NC	Gatehouse et al., 1984

NR = Not reported; NC = no comment; NA = not applicable

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06/05/86

5.4. OTHER REPRODUCTIVE EFFECTS

Topham (1980, 1981) injected male mice intraperitoneally with benzidine at 0, 25, 50, 100 or 250 mg/kg/day for 5 days and observed a >1.7% increase in sperm abnormalities at 50 mg/kg/day compared with controls ($p<0.05$). No increase, however, was observed at 100 or 250 mg/kg/day. Wyrobek et al. (1981) found no significant increase in sperm abnormalities in mice injected intraperitoneally with ≤440 mg/kg/day for 5 days.

5.5. CHRONIC TOXICITY

Several carcinogenicity studies discussed in Section 5.1. also reported non-neoplastic effects of exposure to benzidine.

Littlefield et al. (1983, 1984) gave groups of 72-120 male mice of two strains and 72-120 female mice of two strains 0, 30, 40, 60, 80, 120 or 160 ppm and 0, 20, 30, 40, 60, 80 or 120 ppm (see Table 5-1 for detailed dose schedule) benzidine dihydrochloride in drinking water, respectively, for ~33 months. Dose-related decreases in body weight gain were observed in all treated groups. Mean survival time was also decreased in a dose-related manner in all treated groups; most of the deaths were caused by tumors. A significant dose-related trend in males of both strains ($p=0.001$) was observed with respect to increased incidence of pigmentation (hemosiderin-laden macrophages) of the spleen. The same effect was noted in females, but the level of significance was 0.007. The incidences of the pigmentation were significantly different from control only for the mice treated at ≥120 ppm. The incidence of liver cytological alterations (acidophilic, basophilic, vacuolated and mixed-cell types) attributed to treatment was increased in a dose-related manner in females of both strains at ≥20 ppm. This effect was not dose-related in males. There was a significant linear trend between dose and incidence of hyperplasia of the bile ducts in both

strains of female mice ($p=0.0006$) at $\geq$40 ppm. The incidence of hyperplasia of the bile duct was increased in F_1 and MC males at 160 ppm, but not at $\leq$120 ppm. At concentrations $\geq$60 ppm in female mice and $\geq$120 ppm in male mice, the incidence of megakaryocytosis of bone marrow increased. A slight dose-related trend for increased incidence of hyperplasia of the bladder epithelium was observed in males of both strains ($p=0.16$) but not in females ($p=0.33$). The incidence of epithelium hyperplasia was increased appreciably only in the male mice at 160 ppm. Vacuolization of the brain was observed at higher incidences in all treated groups compared with controls. The incidence in controls ranged from 0-2%. In each strain and sex, the incidence increased in a dose-related manner from 9-17% at 20 ppm to 17-26% at 60 ppm, but decreased to 4-15% at 160 ppm. Since there was a dose-related increase in mortality, the high-dose mice may not have lived long enough to develop this lesion. Atrophy of the ovaries ranged from 9-11% in controls. The incidence increased to 31% at 160 ppm in F_1 mice. In MC mice, the incidence increased to 21% at 60 ppm but decreased to 16% at 120 ppm.

Frith et al. (1979, 1980) exposed groups of male and female weanling mice to drinking water containing 0, 30, 60, 120, 200 or 400 ppm benzidine dihydrochloride for 40, 60 or 80 weeks (see Section 5.1.). Mortality data were not reported. More hepatocellular basophilic and acidophilic foci, hepatocellular adenomas and carcinomas were found in female mice than in males. The lesions were observed at all exposure levels. Because the cell alterations and the tumors occurred in the same mice and because of the time of appearance of the liver lesions, it appeared that the hepatocellular alterations represented preneoplastic lesions (see Section 5.1.).

Frith and Dooley (1976) gave groups of 24 male and 24 female, 3-week-old Balb/c mice benzidine dihydrochloride in their drinking water at levels of 0, 50, 150, 300 and 500 ppm for 8, 19 and 39 weeks (see Section 5.1.). Mortality data were not reported. In addition to preneoplastic and neoplastic changes in the livers of mice exposed to ≥300 ppm, a dose-related increase in the incidence and severity of mild periportal fibrosis, infiltration of lymphocytes and deposits of brown pigment suggestive of ceroid in the liver was observed presumably at ≥50 ppm. These changes were most prominent at 39 weeks. Increased hemosiderin in the spleen was observed at 50 ppm after 19 weeks of exposure and at ≥150 ppm after both 8 and 19 weeks of exposure. After 39 weeks of dosing, hemosiderosis of the spleen was decreased at all doses. Focal vacuolization of transitional epithelium of the urinary bladder was observed in one mouse at 50 ppm after 19 weeks, and in unspecified numbers of mice at 150 ppm after 19 and 39 weeks, at 300 ppm after 8, 19 and 39 weeks and at 500 ppm only at 8 weeks. At 39 weeks, the incidence of vacuolization decreased at all concentrations.

Boyland et al. (1954) fed a group of 10 female Wistar rats diets containing 0.017% benzidine by weight (170 ppm) (see Section 5.1.) until a tumor appeared or death occurred. All treated rats died within 93-224 days. Most of the treated rats developed fatty degeneration and regeneration of the bile ducts and fatty degeneration of the liver.

In a study to determine the MTD for long-term cancer studies, a 6-week dose tolerance test was conducted in which unspecified numbers of male and female B6C3F1 mice were fed diets containing benzidine dihydrochloride in concentrations of 100, 200, 400, 600 and 800 ppm (Vesselinovitch et al., 1975; Rao et al., 1971). The endpoints examined were food consumption,

growth rates and clinical signs of toxicity. Gross and microscopic examinations of the major internal organs and tissues were performed. Reduction in body weight was observed in treated mice as early as 2 weeks after feeding began. A significant dose-related weight loss of 5, 10 and 18% of initial body weight was observed in males fed 400, 600 and 800 ppm, respectively, by the end of the experiment. A less pronounced weight loss of 7.5% was observed in females at the highest dose. A negative linear correlation between doses fed and body weights was observed in both sexes. There was a dose-related decrease in carcass, liver and kidney weights, and a dose-related increase in absolute spleen and thymus weight. Dose-related increased severity of histopathological lesions included cloudy swelling of the liver, vacuolar degeneration of the renal tubules and hyperplasia of myeloid elements in the bone marrow and lymphoid cells in the spleen and thymic cortex. It was not clear if these dose-related effects were observed at all doses.

Zabezhinskii (1970) exposed a group of 48 noninbred albino rats of both sexes, weighing 100-120 g, to benzidine by inhalation at a concentration of 10-20 mg/m^3 (0.755-1.5 mg/kg/day), 4 hours/day, 5 times/week for up to 28 months. Rats that died early in the experiment had focal hepatocellular degeneration and necrosis. Later, regeneration and hyperplasia of Kupffer's cells were observed. In rats that died or were killed at 11 months, fibrosis of the liver became apparent. Thus, inhalation exposure of rats to benzidine appeared to result in cirrhosis. As discussed in Section 5.1., beginning at 13 months, the rats developed myeloid leukemia and mammary gland tumors; only one rat had a hepatoma.

5.6. OTHER RELEVANT INFORMATION

Horton and Bingham (1977) studied the role of the immune system in the same group of benzidine-exposed workers studied by Zavon et al. (1973) (see Section 5.1.2.). In general, low serum properdin levels were found in workers diagnosed with bladder cancer. Workers with high serum properdin levels tended to have benign tumors.

Workers exposed to benzidine may develop dermatitis (Schwartz et al., 1947). The sensitivity of the individual plays a major role in this condition.

Christopher and Jairam (1970) observed low grade glomerulonephritis in benzidine-fed rats. The oral LD_{50}s for benzidine in rats and mice are 309 and 110 mg/kg, respectively; the intraperitoneal LD_{50} in mice is 110 mg/kg (NIOSH, 1985).

5.7. SUMMARY

The carcinogenicity of benzidine has been studied in numerous bioassays in a variety of mammalian species with consistently positive results. Dose-related increased incidences of hepatocellular carcinomas were observed in male and female mice exposed to benzidine dihydrochloride in the drinking water at levels $\geq$20 ppm for up to 33 months (Littlefield et al., 1983, 1984; Frith et al., 1979, 1980). Female mice were more susceptible than male mice. Higher incidences of Harderian gland tumors in both sexes and uterine angiomas in female mice were also observed at all drinking water levels (Littlefield et al., 1983). Liver tumors in mice exposed to benzidine dihydrochloride in drinking water at 300 or 500 ppm were observed as early as 39 weeks of treatment (Frith and Dooley, 1976). Dose-related increased incidences of liver and Harderian gland tumors were also observed in mice exposed to benzidine dihydrochloride in the diet at 50-150 ppm for 90 weeks

(Vesselinovitch et al., 1975). Mice gavaged twice weekly with doses equivalent to the 50 and 100 ppm dietary levels developed fewer liver and Harderian gland tumors than did the mice exposed continuously in the diet. Higher incidences of liver tumors were found in mice when the onset of treatment occurred during infancy than during adulthood. Male offspring of female mice exposed to dietary benzidine dihydrochloride during gestation or lactation had increased incidences of liver tumors, while female offspring did not (Vesselinovitch et al., 1979). Exposure of mouse dams during gestation and lactation, however, resulted in increased incidences of liver tumors in both male and female offspring, but the incidences were higher in males than in females. Thus, male mice appear to be more susceptible than females to the hepatocarcinogenicity of benzidine when exposed early in life, while female mice are more susceptible than males when exposure occurs post-weaning.

Dose-related increased incidences of mammary gland tumors were observed in female rats given 10 doses of 12-50 mg/rat benzidine by gavage over 30 days and observed for 9 months (Griswold et al., 1968). Male and female rats exposed by inhalation to 10-20 mg/m³ benzidine, 4 hours/day, 5 days/week for 28 months also developed mammary gland tumors as well as myeloid leukemia (Zabezhinskii, 1970). Hamsters given benzidine or benzidine dihydrochloride in the diet at 1000 ppm had high incidences of benign and malignant cholangiomas and hepatomas, while hamsters maintained on control diets had no liver tumors (Saffiotti et al , 1967; Sellakumar et al., 1969). Three of six mongrel female dogs treated orally with benzidine in capsules at a TWA dose of 236 mg/kg/day for 60 months and observed for at least 10 years developed urinary bladder cancer (Spitz et al., 1950; Spitz, n.d.).

No bladder tumors, however, were found in dogs fed a total of 700 g of benzidine over 5 years (Bonser et al., 1956), 1.0 mg/kg, 5 days/week for 3 years (Deichmann et al., 1965) or 5 mg/kg/day for 6 years (Marhold et al., 1967). The results indicate that while benzidine may induce bladder cancer in dogs, a long latency period is required.

Numerous epidemiology and case studies have demonstrated that occupational exposure to benzidine results in bladder cancer in humans. Zavon et al. (1973) found 13 cases of bladder cancer in 25 workers exposed to workroom levels of benzidine of <0.005-17.6 mg/m^3 for a mean of 11.46 years. Based on mean urinary levels of benzidine at the end of the workshift, U.S. EPA (1980a) estimated a mean total accumulated dose of 130 mg/kg in the workers. Tsuchiya et al. (1975) reported 72 cases of bladder cancer in 1015 workers engaged in the production or use of benzidine for ≤23 years. Exposure levels were not quantitated. Excess cases of bladder cancer attributable to benzidine exposure have also been reported by Case et al. (1954), Mancuso and El-Attar (1966, 1967), Yamaguchi et al. (1982), Morinaga et al. (1982), Budnick et al. (1984), Segnan and Tonturri (1976), Qi et al. (1983) and Goldwater et al. (1965), among others.

Benzidine has been widely studied for mutagenicity and genotoxicity in bacterial and yeast systems, in cultured mammalian cell systems and in rodents *in vivo*. Results were generally positive (see Section 5.2. and Table 5-13). Although pertinent data regarding the teratogenicity of benzidine to laboratory animals were not available, Vesselinovitch et al. (1979) observed an increased incidence of hepatocellular carcinomas in the male, but not the female, offspring of female mice maintained on a diet containing 150 ppm benzidine dihydrochloride during gestation. Budnick et al. (1984)

found no increased incidence of birth defects in residents of an area contaminated with β-naphthylamine, benzidine and benzene. Topham (1980, 1981) reported an increased incidence of sperm abnormalities in male mice injected intraperitoneally with benzidine at 50 mg/kg/day, but not at 100 or 250 mg/kg/day for 5 days. Wyrobek et al. (1981), however, found no significant increase in sperm abnormalities in mice similarly treated with ≤440 mg/kg/day for 5 days.

In mice exposed to benzidine dihydrochloride in the drinking water at 20-160 ppm for 33 months, there were dose-related decreases in body weight gain and survival at all levels; most deaths were caused by tumors (Littlefield et al., 1983, 1984). The incidence of hemosiderosis of the spleen was significantly increased in mice at ≥120 ppm. Other treatment-related effects included increased incidences of liver cell alterations in females at ≥20 ppm, bile duct hyperplasia in females at ≥40 ppm and in males at 160 ppm, megakaryocytosis of bone marrow in females at ≥60 ppm and in males at ≥120 ppm, bladder epithelial hyperplasia in males at 160 ppm, atrophy of the ovary in females at ≥60 ppm and brain vacuolization in females at ≥20 ppm and in males at ≥30 ppm.

Mice exposed to benzidine dihydrochloride in the drinking water at 50-500 ppm for up to 39 weeks had an increased incidence of mild periportal fibrosis, lymphocytic infiltration and brown pigmentation in the liver, hemosiderosis of the spleen and focal vacuolization of the urinary bladder epithelium at ≥50 ppm.

Dietary administration of benzidine dihydrochloride at 100-800 ppm for 6 weeks resulted in a dose-related loss of body weight of mice at ≥400 ppm (Rao et al., 1971; Vesselinovitch et al., 1975). Dose-related decreases in carcass, liver and kidney weights, dose-related increases in absolute spleen

and thymus weights and dose-related increased severity of cloudy swelling of the liver, vacuolar degeneration of the renal tubules and hyperplasia of myeloid elements were also observed, but the doses were not specified (Rao et al., 1971).

Rats exposed to benzidine in the diet at 170 ppm died within 93-224 days and had fatty degeneration and regeneration of the bile ducts and fatty degeneration of the liver (Boyland et al., 1954). Rats exposed by inhalation to 10-20 mg/m³ benzidine, 4 hours/day, 5 days/week for up to 28 months had liver lesions suggestive of cirrhosis (Zabezhinskii, 1970).

The oral LD_{50} of benzidine is 309 mg/kg in rats and 110 mg/kg in mice (NIOSH, 1985).

6. AQUATIC TOXICITY

6.1. ACUTE

The available information concerning acute toxicity of benzidine to aquatic organisms is presented in Table 6-1. Of the fish species tested, the red shiner, Notropis lutrensis, is the most sensitive, with a 96-hour LC_{50} of 2.5 mg/ℓ (U.S. EPA, 1980a). The least sensitive fish species was the marine sheepshead minnow, Cyprinodon variegatus, with an LC_{50} value of 64 mg/ℓ (Martin, 1982). The most sensitive invertebrate was Daphnia sp., having an EC_{50} value of 1.1 mg/ℓ (Klein et al., 1984).

6.2. CHRONIC

The only study dealing with long-term sublethal effects of benzidine was that of Martin (1982), which was available as an abstract and thus cannot be described in detail. In this study of unspecified duration, sheepshead minnows exposed to "weekly contaminations" of 1 mg/ℓ benzidine developed proliferative liver lesions. Exposure of early embryos to 50 mg/ℓ resulted in heart and head abnormalities. A more detailed description of this study was provided by Courtney and Couch (1984). Sheepshead minnows were exposed to 1 ppm benzidine dihydrochloride in exposures pulsed at 12-day intervals for $\geq$166 days. Several fish became moribund at 166 days and histological examinations revealed liver lesions involving tubular prolification.

6.3. PLANTS

Fitzgerald et al. (1952) reported that 20 mg/ℓ caused a 50% kill of the blue-green alga, Microcystis aeruginosa.

TABLE 6-1

Acute Toxicity of Benzidine to Aquatic Animals

Species	Concentrations (mg/ℓ)	Effect	Reference
		FISH	
Rainbow trout Salmo gairdneri	7.4	96-hour LC_{50}	U.S. EPA, 1980a
Lake trout Salvelinus namaycush	4.35	96-hour LC_{50}	U.S. EPA, 1980a
Red shiner Notropis lutrensis	2.5	96-hour LC_{50}	U.S. EPA, 1980a
Flathead minnow Pimephales promelas	>20	96-hour LC_{50}	U.S. EPA, 1980a
Flagfish Jordanella floridae	16.2	96-hour LC_{50}	U.S. EPA, 1980a
Sheepshead minnow Cyprinodon variegatus	64	LC_{50}, unspecified time	Martin, 1982
Himedaka Oryzias latipes	16.5 10.5	24-hour LC_{50} 48-hour LC_{50}	Tonogai et al., 1982
		CRUSTACEANS	
Waterflea Daphnia sp.	1.1	EC_{50}, unspecified effect	Klein et al., 1984
Scud Gammarus pseudolimnaeus	>20	96-hour LC_{50}	U.S. EPA, 1980a

6.4. RESIDUES

In a study of uptake and metabolism of ^{14}C-labeled benzidine by aquatic organisms, Lu et al. (1977) found that benzidine was rapidly metabolized. Of the total radioactivity present in aquatic species after 3 days exposure to 0.0031 mg/ℓ, the parent compound accounted for 8% in the alga, Oedogonium cardiacum, 12% in mosquitofish, Gambusi affinis, and 13% in the snail Physa sp. The degradation of benzidine in vivo involved N-methylation and acetylation of amino groups. Because benzidine was biodegradable and was not rapidly absorbed, the amount present in organisms was approximately in equilibrium with that in the water. In a laboratory model ecosystem study, biomagnification of benzidine was relatively low, presumably because of the rapid metabolism of benzidine in vivo. Biomagnification factors were as follows: mosquitofish 55; Daphnia magna, 293; mosquito, Lulex pipiens larvae 456; snail 645; and alga, Oedogonium cardiacum 2617. Based on these data, Lu et al. (1977) concluded that benzidine is not bioaccumulated or biomagnified through food chains to high levels. Freitag et al. (1984) and Klein et al. (1984) reported a 1-day BCF of 850 for the alga Chlorella fusca and a 3-day BCF of 83 for the fish Leuciscus idus. EG&G Bionomics (1975) determined that the BCF for benzidine in the edible portion of bluegill, Lepomis macrochirus, was 38-44, and that the BCF for nonedible portions (viscera) was 12-43 times greater. EG&G Bionomics (1975) also reported that the half-life of benzidine in bluegills was ~7 days.

6.5. OTHER RELEVANT INFORMATION

Pliss and Khudoley (1975) fed guppies, Poecilia reticulata and zebrafish, Brachydanio rerio, diets containing 300 ppm benzidine for 56 weeks. No treatment-related tumors were observed, but histopathological liver changes did occur. Khudoley (1977) gave grass frogs, Rana temporaria,

subcutaneous injections and oral doses of benzidine for up to 32 weeks, causing liver and hematopoietic system tumors in 47% of the treated frogs.

6.6. SUMMARY

The available information indicates that benzidine is acutely toxic to fishes at concentrations as low as 2.5 mg/ℓ, the 96-hour LC_{50} for the red shiner (U.S. EPA, 1980a). A concentration of 1 mg/ℓ has been reported to cause sublethal effects in sheepshead minnows (Martin, 1982) and Daphnia sp. (Klein et al., 1984). Data from the studies of Lu et al. (1977) indicate that benzidine is not strongly bioaccumulated by aquatic organisms or biomagnified in aquatic food chains.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

Many agencies, administrations and organizations consider benzidine to be a carcinogen (OSHA, 1984; ACGIH, 1980, 1985; NIOSH, 1978; U.S. EPA, 1980a). Absorption through the skin contributes to the overall exposure to the chemical (ACGIH, 1985); ACGIH (1980) recommended no exposure to or contact with benzidine by any route. U.S. EPA (1980a) recommended zero exposure to benzidine based on the nonthreshold assumption, but recommended criteria of 1.2, 0.12 and 0.01 ng/ℓ corresponding to incremental increased lifetime cancer risks of 10^{-5}, 10^{-6} and 10^{-7}, respectively.

7.2. AQUATIC

Using data on biodegradation, bioaccumulation and acute toxicity to Daphnia sp., Klein et al. (1984) classified benzidine as a class II chemical, i.e., one that has an uncertain likelihood of environmental hazard. U.S. EPA (1980a) reported that 2.5 mg/ℓ was the lowest concentration at which adverse effects on aquatic organisms have been observed.

8. RISK ASSESSMENT

The carcinogenicity of benzidine has been studied in numerous bioassays in a variety of mammalian species with consistently positive results. Dose-related increased incidences of hepatocellular carcinomas were observed in male and female mice exposed to benzidine dihydrochloride in the drinking water at levels ≥20 ppm for up to 33 months (Littlefield et al., 1983, 1984; Frith et al., 1979, 1980). Female mice were more susceptible than male mice. Higher incidences of Harderian gland tumors in both sexes and uterine angiomas in female mice were also observed at all drinking water levels (Littlefield et al., 1983). Liver tumors in mice exposed to benzidine dihydrochloride in drinking water at 300 or 500 ppm were observed as early as 39 weeks of treatment (Frith and Dooley, 1976). Dose-related increased incidences of liver and Harderian gland tumors were also observed in mice exposed to benzidine dihydrochloride in the diet at 50-150 ppm for 90 weeks (Vesselinovitch et al., 1975). Mice gavaged twice weekly with doses equivalent to the 50 and 100 ppm dietary levels developed fewer liver and Harderian gland tumors than did the mice exposed continuously in the diet. Higher incidences of liver tumors were found in mice when the onset of treatment occurred during infancy than during adulthood. Male offspring of female mice exposed to dietary benzidine dihydrochloride during gestation or lactation had increased incidences of liver tumors, while female offspring did not (Vesselinovitch et al., 1979). Exposure of mouse dams during gestation and lactation, however, resulted in increased incidences of liver tumors in both male and female offspring, but the incidences were higher in males than in females. Thus, male mice appear to be more susceptible than

females to the hepatocarcinogenicity of benzidine when exposed early in life, while female mice are more susceptible than males when exposure occurs postweaning.

Dose-related increased incidences of mammary gland tumors were observed in female rats given 10 doses of 12-50 mg/rat benzidine by gavage over 30 days and observed for 9 months (Griswold et al., 1968). Male and female rats exposed by inhalation to 10-20 mg/m³ benzidine, 4 hours/day, 5 days/week for 28 months also developed mammary gland tumors as well as myeloid leukemia (Zabezhinskii, 1970). Hamsters given benzidine or benzidine dihydrochloride in the diet at 1000 ppm had high incidences of benign and malignant cholangiomas and hepatomas, while hamsters maintained on control diets had no liver tumors (Saffiotti et al., 1967; Sellakumar et al., 1969). Three of six mongrel female dogs treated orally with benzidine in capsules at a TWA dose of 236 mg/kg/day for 60 months and observed for at least 10 years developed urinary bladder cancer (Spitz et al., 1950; Spitz, n.d.). No bladder tumors, however, were found in dogs fed a total of 700 g of benzidine over 5 years (Bonser et al., 1956), 1.0 mg/kg, 5 days/week for 3 years (Deichmann et al., 1965) or 5 mg/kg/day for 6 years (Marhold et al., 1967). The results indicate that while benzidine may induce bladder cancer in dogs, a long latency period is required.

Numerous epidemiology and case studies have demonstrated that occupational exposure to benzidine results in bladder cancer in humans. Zavon et al. (1973) found 13 cases of bladder cancer in 25 workers exposed to workroom levels of benzidine of <0.005-17.5 mg/m³ for a mean of 11.46 years. Based on mean urinary levels of benzidine at the end of the workshift, U.S. EPA (1980a) estimated a mean total accumulated dose of 130 mg/kg in the workers. Tsuchiya et al. (1975) reported 72 cases of bladder cancer in 1015

workers engaged in the production or use of benzidine for ≤23 years. Exposure levels were not quantitated. Excess cases of bladder cancer attributable to benzidine exposure have also been reported by Case et al. (1954), Mancuso and El-Attar (1966, 1967), Yamaguchi et al. (1981), Morinaga et al. (1982), Budnick et al. (1984), Segnan and Tonturri (1976), Qi et al. (1983), Goldwater et al. (1965), and Meigs et al. (1986).

Benzidine has been widely studied for mutagenicity and genotoxicity in bacterial and yeast systems, in cultured mammalian cell systems and in rodents *in vivo*. Results were generally positive (see Section 5.2. and Table 5-13). Although pertinent data regarding the teratogenicity of benzidine to laboratory animals were not available, Vesselinovitch et al. (1979) observed an increased incidence of hepatocellular carcinomas in the male, but not the female, offspring of female mice maintained on a diet containing 150 ppm benzidine dihydrochloride during gestation. Budnick et al. (1984) found no increased incidence of birth defects in residents of an area contaminated with β-naphthylamine, benzidine and benzene. Topham (1980, 1981) reported an increased incidence of sperm abnormalities in male mice injected intraperitoneally with benzidine at 50 mg/kg/day, but not at 100 or 250 mg/kg/day for 5 days. Wyrobek et al. (1981), however, found no significant increase in sperm abnormalities in mice similarly treated with ≤440 mg/kg/day for 5 days.

In mice exposed to benzidine dihydrochloride in the drinking water at 20-160 ppm for 33 months, there were dose-related decreases in body weight gain and survival at all levels; most deaths were caused by tumors (Littlefield et al., 1983, 1984). The incidence of hemosiderosis of the spleen was significantly increased in mice at ≥120 ppm. Other treatment-related effects included increased incidences of liver cell alterations in females

at ≥20 ppm, bile duct hyperplasia in females at ≥40 ppm and in males at 160 ppm, megakaryocytosis of bone marrow in females at ≥60 ppm and in males at ≥120 ppm, bladder epithelial hyperplasia in males at 160 ppm, atrophy of the ovary in females at ≥60 ppm and brain vacuolization in females at ≥20 ppm and in males at ≥30 ppm.

Mice exposed to benzidine dihydrochloride in the drinking water at 50-500 ppm for up to 39 weeks had an increased incidence of mild periportal fibrosis, lymphocytic infiltration and brown pigmentation in the liver, hemosiderosis of the spleen and focal vacuolization of the urinary bladder epithelium at ≥50 ppm.

Dietary administration of benzidine dihydrochloride at 100-600 ppm for 6 weeks resulted in a dose-related loss of body weight of mice at ≥400 ppm (Rao et al., 1971; Vesselinovitch et al., 1975). Dose-related decreases in carcass, liver and kidney weights, dose-related increases in absolute spleen and thymus weights and dose-related increased severity of cloudy swelling of the liver, vacuolar degeneration of the renal tubules and hyperplasia of myeloid elements were also observed, but the doses were not specified (Rao et al., 1971).

Rats exposed to benzidine in the diet at 170 ppm died within 93-224 days and had fatty degeneration and regeneration of the bile ducts and fatty degeneration of the liver (Boyland et al., 1954). Rats exposed by inhalation to 10-20 mg/m³ benzidine, 4 hours/day, 5 days/week for up to 28 months had liver lesions suggestive of cirrhosis (Zabezhinskii, 1970).

U.S. EPA (1980a) based its risk assessment on the human epidemiological study by Zavon et al. (1973), which supplied quantitative exposure levels and urinary levels of workers. No other human study provided quantitative exposure data. A few good animal studies post-dating U.S. EPA (1980a) were

located (Littlefield et al., 1983, 1984; Frith et al., 1979, 1980); however, where possible, human data should be used in preference to animal data. Although U.S. EPA (1980a) calculated a criterion based on the study in rats by Griswold et al. (1968) and found it more conservative than the criterion based on the Zavon et al. (1973) study. In these calculations the increase in cancer incidence with age ($-Bd^t$) was estimated to vary by the third power of age. For bladder cancer, however, the rate may be increased by the sixth power of age (Doll, 1971); thus, the q_1^* if recalculated may be approximately double. The latter criterion was still preferred because it seemed reasonable that human epidemiological data were the most appropriate data for estimating human risks. This is further supported by the fact that there is species variation in the metabolism of benzidine. IARC (1982) reviewed the benzidine database and considered the Zavon et al. (1973) epidemiology st dy appropriate for quantitative risk assessment. However, because of the uncertainties relating to exposure estimates and age-related variations in bladder tumor incidence, the U.S. EPA Carcinogen Assessment Group will reevaluate the available information and develop a new quantitative risk estimate if it is determined that such recalculation is warranted. For these reasons, the potency factor (B) of 234.13 $(mg/kg/day)^{-1}$, derived in U.S. EPA (1980a) is recommended as an interim value for estimating the increased lifetime risk of cancer for human exposure to benzidine.

9. REPORTABLE QUANTITY

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

Several studies that were conducted to demonstrate the carcinogenicity of benzidine (see Section 5.1.) also reported non-neoplastic effects (see Section 5.5.), which can be used to derived an RQ for chronic toxicity. These studies are summarized in Table 9-1. Non-neoplastic effects on long-term exposure to benzidine are decreased body weight gain, increased mortality, hemosiderosis of the spleen, bile duct hyperplasia, megakaryocytosis of the bone marrow, hyperplasia of the urinary bladder epithelium, histopathological lesions in the liver and vacuolization of brain cells.

Data used to calculate the CSs are provided in Table 9-2. The MEDs were calculated by multiplying the transformed animal dose by the cube root of the ratio of the animal body weight to the reference human body weight (70 kg), and by 70 kg. As seen from Table 9-1, decreased survival was observed at transformed doses $\geq$1.8 mg/kg/day in the study by Littlefield et al. (1983, 1984). Because the mice were dying from tumors, however, it is not appropriate to calculate a CS for this effect in this study. Decreased survival was also observed in rats at 8.5 mg/kg/day in the Boyland et al. (1954) study. Although some of the rats had tumors, most deaths were due to other causes. Because the study was subchronic, it is necessary to divide the dose by an uncertainty factor of 10, which results in a dose of 0.85 mg/kg/day. The MED is 10.2 mg/day, which corresponds to an RV_d of 4.0. Increased mortality warrants an RV_e of 10. Multiplying the RV_d by the RV_e results in a CS of 40, which corresponds to an RQ of 100.

Cirrhosis of the liver occurred in rats exposed to benzidine by inhalation at an equivalent dose of 0.76-1.5 mg/kg/day (Zabezhinskii, 1970).

TABLE 9-1

Toxicity Summary for Benzidine

Route	Species/ Strain	Sex	Number at Start	Average Weight (kg)	Vehicle Physical State	Compound/ Purity	Exposure/ Duration	Transformed Animal Dose (mg/kg/day)	Response	Reference
Oral	mouse/F_1 or MC[a]	M,F	72-120/ sex/group	~0.035 (M)[b] ~0.027 (F)[b]	drinking water	benzidine •2 HCl/100%	30-160 ppm (3.5-16.6 mg/kg/day) (M); 20-120 ppm (2.5-22.3 mg/kg/day) (F)[b] for 33 months	2.5-119 (M) 1.8-16.0 (F)[c]	Dose-related decreased mean body weight and decreased survival of both sexes of both strains at transformed doses ≥1.8 mg/kg/day (deaths due to tumors); spleen pigmentation at ≥9.5 (M) and ≥10.6 (F) mg/kg/day; bile duct hyperplasia at ≥11.7 (M) and ≥5.6 (F) mg/kg/day; bone marrow megakaryocytosis at ≥9.8 (M) and ≥3.7 (F) mg/kg/day; bladder epithelial hyperplasia at ≥11.7 mg/kg/day (M); mild liver cell alterations at ≥1.8 mg/kg/day (F); brain cell vacuolization at ≥1.8 (F) and >2.5 (M) mg/kg/day	Littlefield et al., 1983, 1984
Oral	mouse/F_1 or MC[a]	M,F	72-216/ sex/group	0.03[d]	drinking water	benzidine •2 HCl/100%	30-400 ppm (5.7-76.0 mg/kg/day)[e] for up to 80 weeks	4.1-54.4[c]	Increased incidences of hepatocellular alterations (acidophilia, basophilia and vacuolization), probably preneoplastic at all doses	Frith et al., 1980
Oral	mouse/ Balb/c	M,F	24/sex/ group	0.03[d]	drinking water	benzidine •2 HCl/NR	≥50 ppm (≥9.5 mg/kg/day)[e] for up to 39 weeks	≥6.8[c]	Dose-related increased incidence and severity of periportal fibrosis, lymphocytic infiltration and pigmentation in liver; hemosiderosis of spleen; vacuolization of bladder epithelium	Frith and Dooley, 1976
Oral	rat/Wistar	F	10	0.35[d]	diet	benzidine/NR	170 ppm up to 224 days	8.5[f]	All rats died within 93-244 days and had fatty degeneration of liver, fatty degeneration and regeneration of bile duct.	Boyland et al., 1954

TABLE 9-1 (cont.)

Route	Species/ Strain	Sex	Number at Start	Average Weight (kg)	Vehicle Physical State	Compound/ Purity	Exposure/ Duration	Transformed Animal Dose (mg/kg/day)	Response	Reference
Inhalation	rat/nonbred albino	M,F	48	0.35[d]	air	benzidine/NR	10-20 mg/m³ 4 hours/day, 5 days/week for up to 28 months	0.76-1.5 g	Cirrhosis of liver	Zabezhinskii, 1970

[a]Strains: F_1 hybrids of BalB/c (male) x C57Bl/6J (female); MC - monohybrid cross offspring of F_1 males x F_1 females

[b]Estimated from data provided by investigators

[c]Calculated by multiplying experimental dose by the ratio of the mol. wt. of the free base to the dihydrochloride

[d]Assumed reference body weights (U.S. EPA, 1980b)

[e]Calculated by assuming that a 0.03 kg mouse drinks 0.0057 L of water/day (Durkin, 1985)

[f]Calculated by assuming that a rat consumes a daily amount of food equal to 5% of its body weight

[g]Calculated by expanding exposure over a 24-hour day and 7-day week, by multiplying by the inhalation rate of a rat (0.223 m³/day) and by dividing by the rat body weight (U.S. EPA, 1980b)

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TABLE 9-2

Composite Scores for Benzidine

Route	Species	Animal Dose (mg/kg/day)	Chronic Human MED (mg/day)	RV_d	Effect	RV_e	CS	RQ	Reference
Oral	mouse	1.8	9.2	4.1	brain cell vacuolization	6	24.6	100	Littlefield et al., 1984
Oral	rat	8.5	10.2*	4.0	decreased survival	10	40	100	Boyland et al., 1954
Inhalation	rat	0.76	9.1	4.1	cirrhosis of liver	7	28.7	100	Zabezhinskii, 1970

*The dose was divided by an uncertainty factor of 10 to approximate chronic exposure.

Using the lower end of the dose range results in an MED of 9.1 mg/day, which corresponds to an RV_d of 4.1. Since cirrhosis indicates a potential decrement of liver function, an RV_e of 7 is warranted. The CS is 28.7, which corresponds to an RQ of 100.

The most severe non-neoplastic effect in the chronic drinking water studies in mice is vacuolization of brain cells in the study by Littlefield et al. (1983,1984). This effect occurred at the lowest dose (1.8 mg/kg/day) in all of the mouse studies; therefore, it is not necessary to calculate CSs for the other effects. Use of the body weight for female mice (0.027 kg) results in an MED of 9.2, which corresponds to an RV_d of 4.1. The RV_e is 6, the CS is 24.6 and the RQ is 100.

Examination of Table 9-2 reveals that all three CS values correspond to RQ values of 100. The highest CS was obtained from the data of Boyland et al. (1954). Of the studies under consideration, Littlefield et al. (1983, 1984) provides the best experimental design. For purposes of determining an RQ based on chronic toxicity, however, the Littlefield et al. (1983, 1984) studies are confounded by the carcinogenic response. Given that most of the rats treated with benzidine in the diet at 8.5 mg/kg/day died from causes other than tumors (Boyland et al., 1954), it is possible that had the mice in the Littlefield et al. (1983, 1984) studies not developed fatal tumors, they may have died from causes that were otherwise obscured by the overwhelming carcinogenic effect. Because of this concern about benzidine-induced mortality, the study by Boyland et al. (1954) is recommended as the basis for the RQ based on the chronic toxicity of benzidine (Table 9-3).

In a previous determination of the RQ, U.S. EPA (1983a) used the study of Frith et al. (1980) as the basis of the RQ (MED = 22.4 mg/day, RV_d = 3.5, RV_e = 8, CS = 28, RQ = 100). This study cannot be recommended as the

TABLE 9-3

Benzidine

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose*:	10.2 mg/day
Effect:	decreased survival
Reference:	Boyland et al., 1954
RV_d:	4.0
RV_e:	10
Composite Score:	40
RQ:	100

*Equivalent human dose

basis for RQ in the current determination for several reasons. The effect was hepatic cellular alteration, which according to the recommended rating scheme, should warrant an RV_e no higher than 6. Furthermore, the lesions appeared to be preneoplastic and, therefore, not appropriate for RQ consideration. The studies by Littlefield et al. (1983, 1984) were not available for consideration. The U.S. EPA (1983a) did consider the study by Boyland et al. (1954); however, no uncertainty factor was used in determining the MED.

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

The evidence that benzidine is carcinogenic to humans and animals is overwhelming. Benzidine dihydrochloride induced hepatocellular carcinomas, Harderian gland tumors and uterine angiomas when administered in the drinking water (Littlefield et al., 1983, 1984; Frith et al., 1979, 1980; Frith and Dooley, 1976), in the diet or by gavage (Vesselinovitch et al., 1975, 1979; Miyakawa and Yoshida, 1980; Osanai, 1976; Ikuyama et al., 1978) to mice. Gavage treatment of rats with benzidine in sesame oil resulted in increased incidences of mammary gland tumors (Griswold et al., 1968). Dietary administration of benzidine or benzidine dihydrochloride to hamsters resulted in high incidences of benign and malignant cholangiomas and hepatomas (Saffiotti et al., 1967; Sellakumar et al., 1969). Dogs fed benzidine in capsules for 5 years developed urinary bladder tumors after long latency periods (Spitz et al., 1950; Spitz, n.d.). Rats exposed by inhalation to benzidine developed myeloid leukemia (Zabezhinskii, 1970). Several of these studies are summarized in Tables 5-1 to 5-9. Studies in which benzidine was administered parentally lend further support. For example, subcutaneous injection has resulted in intestinal tumors in rats (Pliss et al., 1973), mammary tumors in rats (Steinhoff, 1974, 1981), Zymbal gland tumors in rats

(Pliss and Vol'fson, 1972), hepatomas in mice (Baker, 1950; Bonser et al., 1956; Prokof'eva, 1971) and rats (Spitz et al., 1950), adenocarcinomas in mice (Prokof'eva, 1971) lymphoma in mice (Bonser et al., 1956) and tumors of the sebaceous glands of the auditory canal in rats (Spitz et al., 1950). Mammary and Zymbal gland tumors were observed in rats after intraperitoneal injection (Morton et al., 1981).

Numerous epidemiology and case studies have demonstrated that occupational exposure to benzidine results in bladder cancer in humans. Zavon et al. (1973) found 13 cases of bladder cancer in 25 workers exposed to workroom levels of benzidine of <0.005-17.6 mg/m³ for a mean of 11.46 years (see Table 5-10). Based on mean urinary levels of benzidine at the end of the workshift, U.S. EPA (1980a) estimated a mean total accumulated dose of 130 mg/kg in the workers. Tsuchiya et al. (1975) reported 92 cases of bladder cancer in 1015 workers engaged in the production or use of benzidine for ≤23 years (see Table 5-11). Exposure levels were not quantitated. Case et al. (1954) reported that 10 deaths were attributed to bladder tumors in benzidine-exposed workers in the British chemical industry compared with 0.72 expected in the male population of England and Wales (see Table 5-12). Excess cases of bladder cancer attributable to benzidine and/or ß-naphthylamine exposure have also been reported by Mancuso and El-Attar (1966, 1967), Yamaguchi et al. (1982), Morinaga et al. (1982), Budnick et al. (1984), Segnen and Tanturri (1976), Qi et al. (1983) and Goldwater et al. (1965), among others.

Therefore, the data are sufficient from both animal and human studies to classify benzidine as an EPA Group A (U.S. EPA, 1984) and an IARC Group 1 human carcinogen.

As discussed in Chapter 8, U.S. EPA (1980a) calculated a potency factor (B) of 234.13 $(mg/kg/day)^{-1}$ to estimate the increased lifetime risk of cancer for human exposure to benzidine based on the human data reported by Zavon et al. (1973). Dividing 0.1 by (B) results in an ED_{10} of 4.235×10^{-4} mg/kg/day, the reciprocal of which equals an F factor of 2361 $(mg/kg/day)^{-1}$ (Table 9-4). Because the F factor is >100, benzidine is placed in Potency Group 1, which has a HIGH hazard ranking under CERCLA.

In a previous determination of the F factor, (SRC, 1983; U.S. EPA, 1983b), the study of Saffiotti et al. (1967) in the hamster (see Table 5-9) was used as the basis of the F factor. That F factor of 1.9 $(mg/kg/day)^{-1}$ with a medium hazard ranking greatly underestimates the potency of benzidine as a human carcinogen. SRC (1983) apparently overlooked the U.S. EPA (1980a) risk assessment and mistakenly stated that exposure levels were unavailable for the human study by Zavon et al. (1973). The current determination of F appropriately uses the human data.

TABLE 9-4

Derivation of Potency Factor (F) for Benzidine

Reference:	Zavon et al., 1973; U.S. EPA, 1980a
Exposure route:	occupational
Species:	human
Sex:	male
Duration of exposure:	11.46 years
Average age of cohort at end of study:	56.5 years
Lifespan of human:	71.3 years*
Target organ:	bladder
Tumor type:	malignant
Exposure level:	0.005-17.6 mg/m^3
Average lifetime exposure (mg/kg/day):	0.0063*
Tumor incidence:	13/25
B:	234.13 (mg/kg/day)$^{-1}$
ED_{10}:	4.235×10^{-4} mg/kg/day
$1/ED_{10}$ (F factor):	2361 (mg/kg/day)$^{-1}$

*See U.S. EPA, 1980a

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APPENDIX

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

CASR online (U.S. EPA Chemical Activities Status Report)
CAS online STN International
TOXLINE
TOXBACK 76
TOXBACK 65
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP
NTIS
Federal Register

These searches were conducted in October, 1985. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

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Worthing, C.R. and S.B. Walker, Ed. 1983. The Pesticide Manual. British Crop Protection Council. 695 p.

Windholz, M., Ed. 1983. The Merck Index, 10th ed. Merck and Co., Inc., Rahway, NJ.

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

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Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. U.S. Dept. Interior, Fish and Wildlife Serv. Res. Publ. 137, Washington, DC.

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